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THE AMERICAN REVIEW OF TUBERCULOSIS AND PULMONARY DISEASES

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MEDICAL SECTION OF NATIONAL TUBERCULOSIS ASSOCIATION

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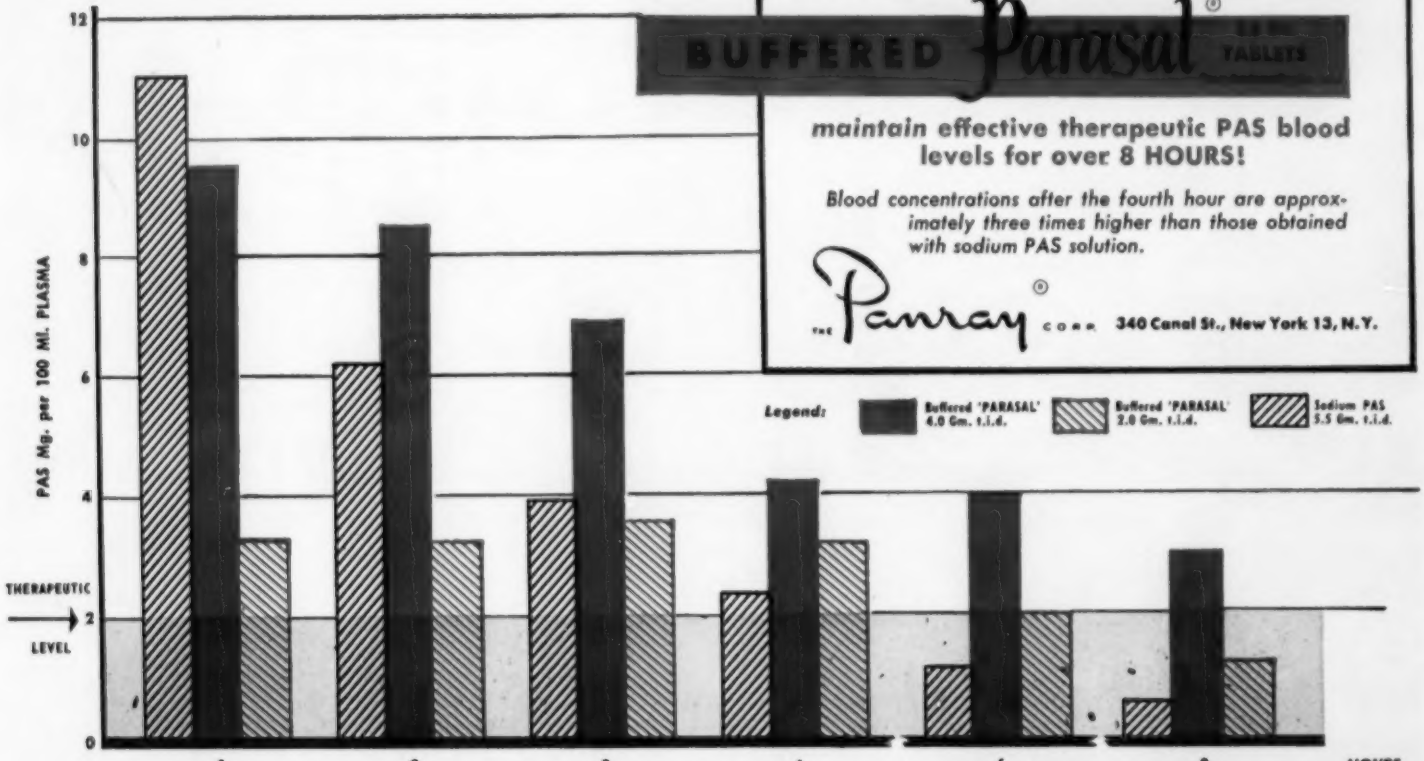

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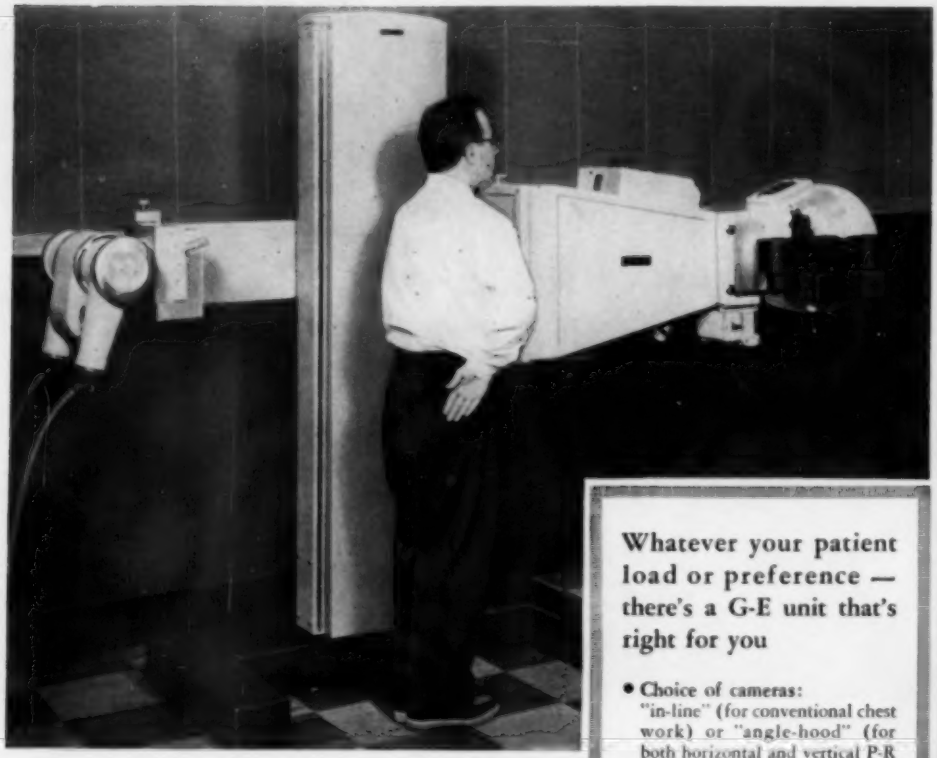

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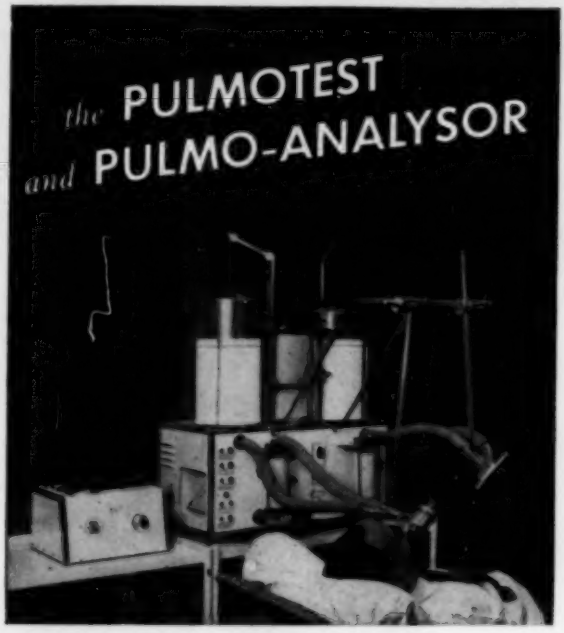

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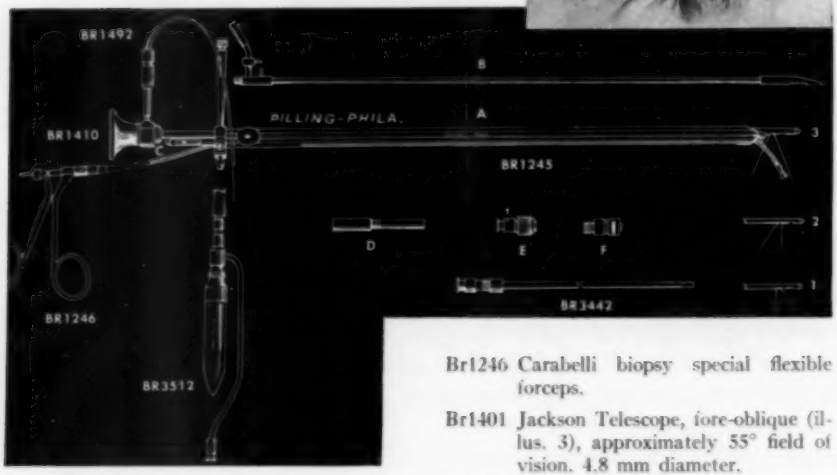

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THE AMERICAN REVIEW OF TUBERCULOSIS AND PULMONARY DISEASES

VOLUME 75 *March, 1957* NUMBER 3

A STUDY COMPARING THE EFFECTS OF BED REST AND PHYSICAL ACTIVITY ON RECOVERY FROM PULMONARY TUBERCULOSIS[1]

JAMES G. HIRSCH, RUSSELL W. SCHAEDLER, CYNTHIA H. PIERCE, AND I. MACLEAN SMITH

(Received for publication December 14, 1956)

Introduction

The beneficial effect of bed rest on recovery from tuberculosis was widely accepted during the era prior to chemotherapy. Although no adequately controlled study was ever done to prove this relationship, the almost unanimous opinion of so many physicians, based on their clinical observations, served to establish bed rest as the mainstay of the therapeutic regimen for phthisis.

Since the introduction of effective chemotherapy for tuberculosis, there seems to be increasing disagreement, both in theory and in practice, concerning the relative role of bed rest. In the opinion of many physicians, physical inactivity still exerts a beneficial effect which is merely masked by the superimposed action of antimicrobial agents. Others suspect that the effect of chemotherapy is so marked in comparison with that of bed rest as to render the latter relatively unimportant. Some have even speculated that bed rest may be antagonistic to optimal action of the antimicrobial drugs.

How much bed rest is necessary for ideal therapy of tuberculosis at the present time? This question is of obvious practical importance both to the individual patient and his physician, and to health officials responsible for planning facilities and procedures for the care of victims of tuberculosis.

The present communication presents results of a controlled study comparing the effects of bed rest and of physical activity on the course of recovery from moderately far advanced or far advanced pulmonary tuberculosis treated with antimicrobial drugs.

Plan of the Study

Therapeutic regimens for tuberculosis can be critically evaluated only if the observations are reliable and suitably controlled. The cooperative type of study used so effectively in recent years for investigating antimycobacterial drugs can be adequately controlled, but it is difficult to keep such large numbers of patients

[1] From the Rockefeller Institute for Medical Research, New York, New York.

under reliable twenty-four-hour observation. Constant observation is essential to a study comparing bed rest and physical activity; unless the investigators can be certain that the prescribed activity or inactivity is rigidly maintained, the results are open to doubt.

Over the course of the past two years, an attempt has been made to assess the role of bed rest in treating a relatively small number of tuberculous patients at the Rockefeller Institute Hospital. Instead of employing the usual method of control, that is, treating alternate patients in different fashion, each patient was alternated through periods of bed rest and of physical activity, and detailed clinical and laboratory observations were made to determine whether or not physical activity would be reflected in clinical worsening, or at least in slowing of the rate of recovery. Thus, in a sense each patient served as her own control. The conditions in this research unit were such that all patients were under almost constant surveillance by a responsible member of the hospital staff, thus assuring that there were no violations of the rest or activity ordered by the physicians.

At the outset of this study, it was anticipated that physical activity might well exert a detrimental effect on recovery from tuberculosis. Caution was used, therefore, and, as will be seen in the charts at the end of the present paper, at first patients were treated with an initial period of bed rest. Moreover, no patient was placed on physical activity until she was afebrile. As results became available, the approach grew progressively more liberal. The final group of patients (*Cases 15* through *21*) was treated with physical activity or bed rest in a predetermined random fashion, with variation in both the duration and the sequence of rest and activity periods.

The patients[2] were all adult females with moderately far advanced or far advanced pulmonary tuberculosis thought to be of recent origin. None gave a history of prior treatment for tuberculosis. They were consecutive admissions except for 2 patients who were eliminated from the study because their pretreatment cultures revealed tubercle bacilli highly resistant to streptomycin, isoniazid, and PAS.[3]

On entering the hospital each patient was placed on bed rest until the diagnosis was established and the preliminary evaluation made. As soon as possible (usually within one week of admission) therapy was started and the study begun.

[2] The patients were obtained for study through the cooperation of the Bureau of Tuberculosis, Department of Health of the City of New York. We are especially grateful to Drs. Aaron D. Chaves, Vera Leites, Louis Schneider, and Emanuel Schwartz for referring suitable cases to us.

[3] These 2 patients with triple resistance both denied previous therapy for tuberculosis. One of them, however, was found to have a record of treatment for tuberculosis in several other hospitals with prolonged administration of all three of the above antimicrobial agents. This individual steadfastly denied receiving prior therapy until she was confronted with the evidence; she then revealed that she had been untruthful because she feared her history would have prevented admission to the hospital. The other patient with triple-drug resistance seemed reliable, and no evidence was found to refute her story. The source of infection was not established.

The antimicrobial regimen employed as a routine consisted of streptomycin sulfate, 1 gm. intramuscularly three times a week, and isoniazid, 5 mg. per kg. of body weight daily. In some patients in whom drug resistance was encountered, PAS was substituted or added. No significant drug toxicity occurred.

No collapse or surgical therapy was employed.

During the bed-rest phase, patients were confined to their beds except for a maximum of four daily brief visits to the lavatory. At mealtimes the bed gatch was elevated; at all other times the patients were required to lie flat in bed. The only activities permitted were reading and listening to the radio.

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9 a.m.–9:30 a.m.: Doctor's rounds.

9:30 a.m.–10 a.m.: Group exercises. These exercises, supervised by the nursing staff, were patterned after calisthenics used in the armed forces. They included such maneuvers as arm movements, trunk flexing, and deep knee bends.

10 a.m.–noon: Unlimited occupational therapy.

Noon–1 p.m.: Lunch at the table and clean-up of dishes and table.

1 p.m.–2 p.m.: Rest in bed.

2 p.m.–3 p.m.: Unlimited occupational therapy.

3 p.m.–5 p.m.: Visiting hours and activity of choice, such as card playing, dancing, watching television, or volley ball.

5 p.m.–6 p.m.: Supper at the table and clean-up of dishes and table.

6 pm.–6:30 p.m.: Group exercises.

6:30 p.m.–7:30 p.m.: Rest in bed.

7:30 p.m.–9:30 p.m.: Activity of choice, including occupational therapy.

9:30 p.m.–10 p.m.: Preparation for bed.

10 p.m.–7 a.m.: Sleep.

Thus, when physical activity was prescribed, the patients were required to be up for twelve hours daily; they were allowed to remain in bed only during the two day-time one-hour rest periods and while sleeping at night.

It was deemed inadvisable to shift patients from bed rest directly into the activity schedule outlined above. Therefore, during the first week of an active phase following one of bed rest, patients were allowed up stepwise, increasing one to two hours daily until full activity was reached.

Clinical evaluation: During the first four weeks of hospitalization, temperatures, pulses, and respirations were recorded every four hours except during the night sleep period. Thereafter, provided the patient was afebrile, temperatures were taken twice daily. In the charts which follow at the end of the paper, fever is indicated by a (+) in any week in which a rectal temperature of 38° C. or higher was obtained on more than one occasion. Sputum was collected and weighed daily. The average daily sputum production for one-week periods is recorded on the summary charts. No hemoptysis was encountered. Each patient was weighed on the same scale and at the same hour of the day once weekly.

The patients were seen daily by their physician, and once a week they were examined by at least two clinicians and the pertinent changes in physical findings were noted. (Since the physical findings in all cases changed in keeping with roentgenographic and other laboratory observations, they have been omitted from the charts for purposes of clarity.)

Laboratory evaluation: On admission postero-anterior, lateral, and tomographic roentgenograms of the chest were obtained. Thereafter postero-anterior chest plates were taken every two weeks. Every two months tomograms were repeated through levels showing major infiltration or cavitation. The chest roentgenograms were interpreted by an outside radiologist unacquainted with the nature of the study as well as by the investigators. Schematic diagrams of the roentgenographic abnormalities are presented in the summary charts. Stippled areas indicate regions of patchy density, cross-hatching represents confluent density, and closed circles show cavities as demonstrated by standard films or tomography.

On admission and every two weeks thereafter, a twenty-four-hour sputum specimen was examined bacteriologically, complete drug-susceptibility studies being done once a month. When sputum was no longer produced, gastric lavage specimens were collected.

All antimicrobial drugs were discontinued for twenty-four hours preceding and the twenty-four hours during collection to avoid possible drug contamination of the samples to be cultured. After digestion at 37°C. for thirty minutes with 3 per cent NaOH, each sample was spun (International size 2 centrifuge at 2,500 r.p.m. for twenty minutes) and the sediment was neutralized with 1N HCl in phenol red. The sediment was then suspended in sterile 0.1 per cent bovine albumin in distilled water and recentrifuged. A portion of the sediment was stained for acid-fast bacilli and the remainder was inoculated onto the surface of three freshly prepared Löwenstein-Jensen slants. The initial sputum concentrate was also injected into two guinea pigs. The following scheme was used for quantitation of positive cultures:

4+ = colonies too numerous to count

3+ = more than 100 colonies total

2+ = 10–100 colonies

1+ = 1–10 colonies

Drug susceptibility was studied by a standard technique described elsewhere (1) and, in some cases, by a slide culture procedure (2).

On admission a complete blood count, erythrocyte sedimentation rate, urinalysis, stool examination, serologic test for syphilis, C-reactive protein determination (3), fasting blood sugar, and Middlebrook-Dubos hemolysin test (4) were done on all patients. Blood counts, C-reactive protein determination, and Westergren sedimentation rate test were repeated at two-week intervals. Serologic tests for tuberculosis were done at approximately two-month intervals, titers being recorded as the reciprocal of the highest dilution of serum producing distinct hemolysis.

With few exceptions, all patients remained in the hospital until three consecutive cultures were negative for tubercle bacilli and the roentgenographic changes

had shown stability. Follow-up examinations, including chest plates and sedimentation rates, were performed at one- to two-month intervals.

Results

The results of this investigation are presented at the end of the paper in the form of summary charts on each patient showing the course of recovery in relation to bed rest and physical activity. Also included are reproductions of the chest roentgenograms taken on admission and after approximately six months' hospitalization.

It is evident from examination of the charts that the course of recovery from tuberculosis in these patients was not detectably different on bed rest or on physical activity.

Some general comments on various clinical and laboratory observations follow:

Fever: Only 4 patients had recurrence of fever during their hospital course. In 2 of these (*Cases 6* and *19*) the recurrent fever was clearly associated with a complicating nontuberculous illness. In *Case 14* the three episodes of low-grade temperature elevation appeared to be unrelated to bed rest or physical activity; in one instance fever began while the patient was at bed rest; in another, during transition from bed rest to activity; and in the third, while on physical activity. Low-grade fever of brief duration, unaccompanied by other signs or symptoms, occurred late in the course of *Case 13* during a period of physical activity.

Sputum production: The quantity of sputum produced daily diminished progressively in all except 3 cases. In one, *Case 11*, the patient again began to expectorate in the seventh month of hospitalization during an overt relapse in her tuberculosis which was coincident with the appearance of isoniazid-resistant bacilli in gastric contents and sputum. In *Cases 14* and *16* sputum production increased slightly for brief periods during the hospital course; in both instances this increase in expectoration appeared while the patients were on bed rest.

Chest roentgenograms: In all cases the appearance of the chest roentgenograms improved gradually; clearing of pulmonary infiltration and closing of cavities were not notably different during periods of bed rest and of physical activity. In no instance did physical activity bring about detectable worsening of the chest roentgenogram.

Weight: The weight of all patients increased during their hospital stay, the magnitude of weight gain ranging from a few to more than 25 kilograms. In many instances the rate of weight increase appeared to be more rapid while the patients were on physical activity than on bed rest (*see Cases 1*, *2*, *9*, and *10*). Physical activity did not retard weight gain of any patient.

Erythrocyte sedimentation rate: The erythrocyte sedimentation rate fell progressively during therapy in most cases and appeared not to be influenced by the cycles of rest and activity. In *Cases 4*, *5*, and *9* the erythrocyte sedimentation rate rose slightly or was stationary during the initial period of physical activity; similar temporary elevations in sedimentation rate occurred in *Cases 1*, *10*, and *14* during the initial or an early period of bed rest. In *Cases 11* and *18* sedimentation rates rose significantly in association with a clinical relapse and a

failure to respond to therapy, respectively. In both of these patients drug-resistant tubercle bacilli were found in the sputum. In *Case 11* the late elevation in erythrocyte sedimentation rate began while the patient was at bed rest, while in *Case 18* it occurred during physical activity. In *Case 11* and *Case 18*, the institution of more appropriate chemotherapy was followed by a falling sedimentation rate while the patients were maintained on physical activity.

C-reactive protein: In confirmation of observations recently reported (5), the C-reactive protein determination served as a simple and reliable index of the clinical progress. Erythrocyte sedimentation rate and C-reactive protein values were usually parallel. C-reactive protein tests became steadily less positive in most patients and appeared not to be different during periods of bed rest and physical activity. Minor temporary elevations in quantity of C-reactive protein were noted in some patients; in *Case 5* such an elevation took place during physical activity and in *Case 6* while the patient was at bed rest. In *Case 11* the C-reactive protein became more positive during a clinical relapse.

Cultures: In almost all patients the cultures of the sputum or gastric washings became progressively less positive for tubercle bacilli. There was no detectable increase in expectoration of mycobacteria associated with physical activity. Five patients became transiently "sputum positive" after having been free of tubercle bacilli on one or more prior cultures; in 3 of these (*Cases 3*, *10*, and *13*) the tubercle bacilli reappeared in the sputum while the patient was at bed rest, and in 2 while on physical activity (*Cases 11* and *14*).

In only one patient (*Case 11*) did drug-resistant tubercle bacilli appear during therapy. However, in 5 of the 21 patients (*Cases 9*, *10*, *18*, *19*, and *21*) drug-resistant bacilli were cultured prior to starting chemotherapy. Taking into account the 2 patients eliminated from the study because of multiple-drug resistance (*see* Plan of Study section), tubercle bacilli resistant to streptomycin, isoniazid, or PAS were isolated from 7 of 23 consecutive patients at the time of admission to the hospital. Only one of these 7 patients was known to have received prior chemotherapy. This high incidence of apparent infection with drug-resistant tubercle bacilli is in contrast to the observations of Chaves and associates (6) and may reflect chance selection in a relatively small number of patients.

Hemolysin titers: The hemolytic modification of the Middlebrook-Dubos serologic test for tuberculosis was of little aid in following the course of the disease. All patterns were observed: elevated relatively stable titers throughout the hospital course, high titers on admission which returned towards normal during recovery, and low titers on admission rising during recovery. There was no apparent correlation of the hemolysin titer pattern with the clinical course of the infection.

Discussion

The data obtained in this study suggest that recovery from fresh pulmonary tuberculosis treated with modern chemotherapy is not significantly different in patients at bed rest or on physical activity. The question immediately arises

whether the technique for following the clinical course was sufficiently sensitive to have revealed a deleterious effect of physical activity had there been one. Although no conclusive evidence can be presented to answer this question, the charts of some of the patients infected with drug-resistant tubercle bacilli provide helpful information. In *Case 11*, for example, the development of drug resistance approximately five months after the patient had been admitted to the hospital was well correlated with clinical and laboratory signs of worsening: fever, increasing rales, reappearance of tubercle bacilli in the sputum, rising erythrocyte sedimentation rate, and increase in C-reactive protein. Similar findings are demonstrated in *Case 18*, although, in this instance, drug resistance was present on admission, and the course of recovery was slow and erratic until PAS was added to the therapeutic regimen. Thus the technique for following the course was sufficiently sensitive to reflect clearly a lack of response or a relapse associated with inappropriate drug therapy. It follows, therefore, that, had there been significant harmful effects resulting from physical activity, the methods for observing the patients should have revealed it.

This study was designed to test the effect on recovery from tuberculosis of only one variable—bed rest as compared with physical activity. It is unwarranted to consider that the results have significance for the *home* treatment of tuberculosis. Especially in the case of patients from homes filled with economic, social, and psychologic problems, there are obviously many differences between home and hospital other than the amount of physical rest or activity. It remains to be demonstrated whether or not these other factors play a significant role in influencing the response of patients under effective chemotherapy. The recent study of Tyrrell (7), comparing the course of patients under chemotherapy in the hospital and at home suggests that, although the course of recovery is in general similar under these circumstances, cavity closure may be delayed or impaired in tuberculous patients treated at home as compared with the results in similar patients treated in the hospital.

The results presented in this communication deal only with relatively short-term recovery from tuberculosis. Further studies are needed before it will be possible to assess the influence, if any, of physical activity on the incidence of relapse. The over-all results of treatment in this series of cases appear to be quite good. All patients were brought to a "sputum negative" state and all cavities were closed in so far as could be demonstrated by tomography. Considering the severity of the disease treated, this record seems an excellent one. However, the number of cases is so small, and the duration of follow-up so short, that these observations justify only tentative conclusions. All patients have been examined at frequent intervals since discharge from the hospital, and to date there has been only one confirmed relapse. This occurred in a patient, *Case 15*, who left the hospital against advice after only four months of treatment and who failed to take her medication thereafter.

It is important to point out that the results presented here may pertain only to relatively fresh previously untreated pulmonary tuberculosis in adult females. Further studies are required to determine whether or not bed rest significantly

affects recovery in patients of different age and sex and in persons afflicted with other types of tuberculosis.

For many years now, most physicians have considered bed rest the *sine qua non* of tuberculosis therapy. Indeed, their respect for bed rest often amounts to a reverence. Perhaps this feeling was warranted prior to the introduction of effective chemotherapy. To continue dogmatically to hold such an attitude at present seems unwise. Bed rest is potentially harmful treatment; the unnecessary prescription of prolonged inactivity is as dangerous and unjustified as is, for example, the use of a potentially toxic drug or a crippling operative procedure without due cause. Prolonged bed rest often results in harmful metabolic and psychologic effects which cannot be disregarded. It is to be hoped that additional clinical investigations in the near future will provide the evidence needed to define objectively the role of bed rest in the modern treatment of tuberculosis.

Summary

The effects of bed rest and of physical activity on recovery from tuberculosis have been compared in controlled fashion. Adult women afflicted with moderately far advanced or far advanced relatively fresh pulmonary tuberculosis were subjected to alternating periods of bed rest and of supervised physical activity while under treatment with combined chemotherapy. Detailed clinical and laboratory observations revealed no detectable deleterious effect of physical activity on the course of recovery.

Sumario

Estudio Comparativo de los Efectos del Descanso en Cama y de la Actividad Física sobre la Reposición de la Tuberculosis Pulmonar

Se han comparado en forma fiscalizada los efectos del reposo en cama y de la actividad física sobre la curación de la tuberculosis. Mujeres adultas afectadas por tuberculosis pulmonar moderadamente avanzada o relativamente reciente muy avanzada fueron sometidas a períodos alternados de descanso en cama y de actividad física vigilada mientras recibían tratamiento con quimioterapia combinada. Minuciosas observaciones clínicas y de laboratorio no revelaron el menor efecto nocivo distinguible de la actividad física sobre la marcha de la reposición.

Resume

Une étude comparative des effets de l'alitement et de l'activité physique sur la guérison de la tuberculose pulmonaire

Les effets de l'alitement et ceux de l'activité physique sur la guérison de la tuberculose pulmonaire ont été confrontés de façon contrôlée. Des adultes de sexe féminin atteintes de tuberculose pulmonaire relativement récente, modérément ou très avancée, fuerent soumises à des périodes alternées d'alitement et d'activité physique au cours d'un traitement par la chimiothérapie combinée. Aucun effet nocif de l'activité physique sur le cours de la guérison n'a été révélé par les recherches détaillées qui ont été effectuées en clinique et au laboratoire.

Acknowledgment

The writers acknowledge with gratitude many helpful suggestions from and stimulating discussions with Dr. René J. Dubos and Dr. Frank L. Horsfall, Jr.

REFERENCES

(1) HALL, W. H., RUNYON, E. H., AND BERNSTEIN, S.: Diagnostic laboratory methods as revised following the Twelfth Veterans Administration-Army-Navy-Conference on the Chemotherapy of Tuberculosis, Atlanta, Georgia, February 9–12, 1953.

(2) PIERCE, C. H., HIRSCH, J. G., AND SCHAEDLER, R. W.: Rapid detection of drug-resistant tubercle bacilli in sputum by slide cultures, Am. Rev. Tuberc., 1957, *75*, 331.

(3) ANDERSON, H. C., AND MCCARTY, M.: Determination of C-reactive protein in the blood as a measure of the activity of the disease process in acute rheumatic fever, Am. J. Med., 1950, *8*, 445.

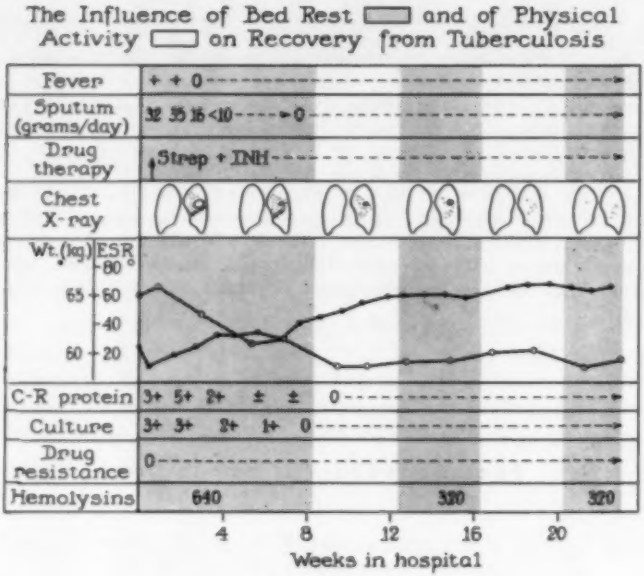
(4) MIDDLEBROOK, G.: A hemolytic modification of the hemagglutination test for antibodies against tubercle bacillus antigen, J. Clin. Investigation, 1950, *29*, 1480.

(5) WALSH, J. J., LINN, R. H., DERBES, V. J., TURNER, M., AND JACOBS, S.: C-reactive protein in pulmonary tuberculosis, Am. Rev. Tuberc., 1956, *74*, 464.

(6) CHAVES, A. D., ROBINS, A., ABELES, H., PEIZER, L. R., DANGLER, G., AND WIDELOCK, D.: Further observations on the prevalence of streptomycin- and isoniazid-resistant strains of *Mycobacterium tuberculosis* in patients with newly discovered and untreated active pulmonary tuberculosis, Am. Rev. Tuberc., 1956, *74*, 293.

(7) TYRRELL, W. F.: Bed rest in the treatment of pulmonary tuberculosis, Lancet, 1956, *1*, 821.

FIGURES FOR *Cases 1–21* WILL FOLLOW



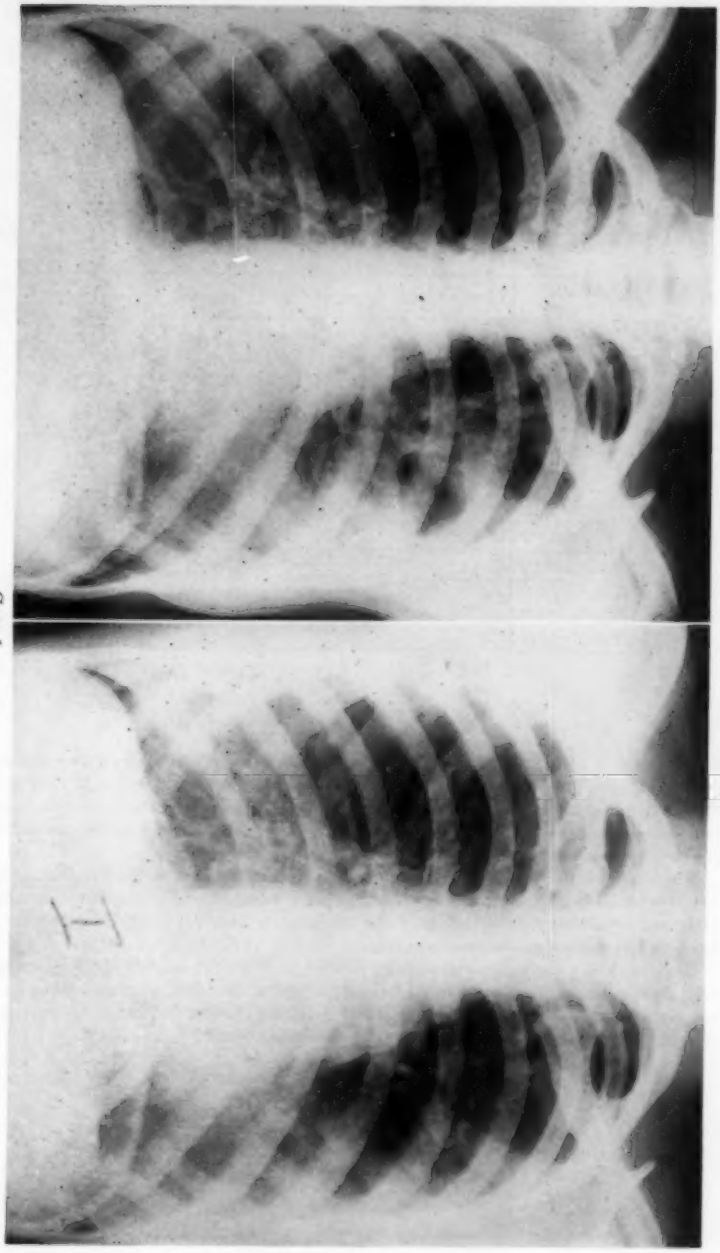
Case 1 (S. B.)

This patient, a 16-year-old Negro girl student, was admitted to the hospital on January 28, 1955, with a history of an illness of one month's duration characterized by evening fever, vague left chest pain, cough productive of non-bloody sputum, and a 10 pound weight loss. There was no known exposure to tuberculosis.

Physical examination revealed a healthy-looking young girl. The only significant abnormality was the presence of rales over the area of the left lower lobe.

Her course was uncomplicated except for the occurrence of inspiratory and expiratory wheezes, sneezing, and nasal discharge in June, 1955. She had suffered an asthmatic attack ten years previously. Symptomatic therapy provided relief during the few weeks of this episode.

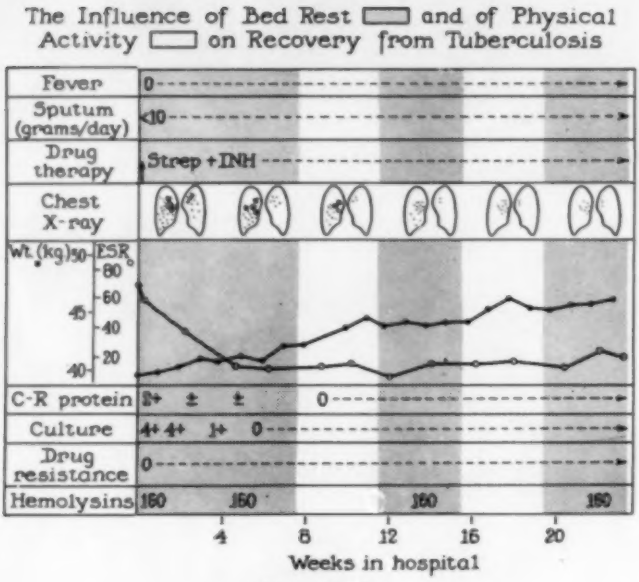
On October 14, 1955, eight and a half months after admission, the patient was discharged from the hospital. The chemotherapy consisted of isoniazid and streptomycin. Streptomycin was continued for one year and the isoniazid is being administered for an indefinite period. In February, 1956, approximately one year after the onset of her illness, she returned to school. Regular examinations in the clinic indicate apparent arrest of the disease.



January 28, 1955

Case 1

July 11, 1955

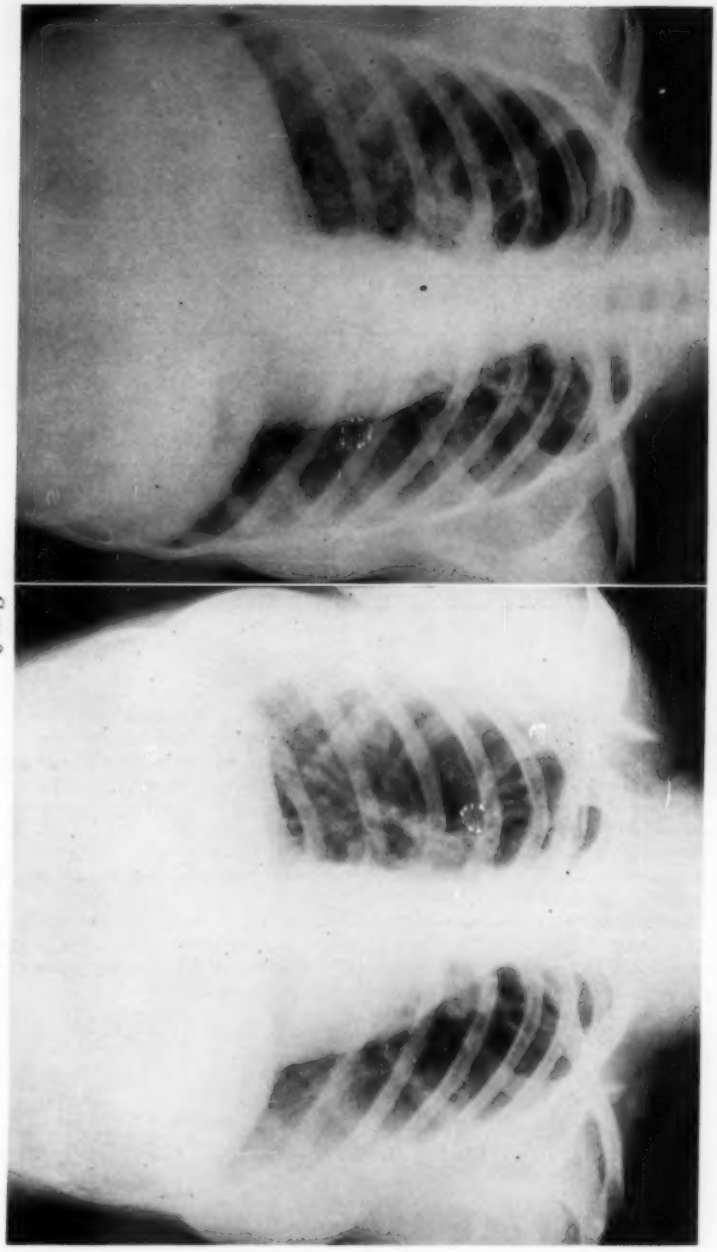


Case 2 (C. C.)

This patient, a 12-year-old Puerto Rican schoolgirl, was admitted to the hospital on February 2, 1955, with a history of chronic productive cough for six months. In 1947 her mother had died from tuberculosis and a baby sister also succumbed to this disease in 1950. The patient was followed in a city chest clinic because of the family history of tuberculosis. A chest roentgenogram in 1954 was apparently normal.

Physical examination showed an unkempt, well-developed, well-nourished girl who did not appear ill. Significant positive findings were rales over all lung fields and an area of increased breath sounds at the tip of the left scapula. Her voice was deep and throaty, but indirect laryngoscopy was nonrevealing.

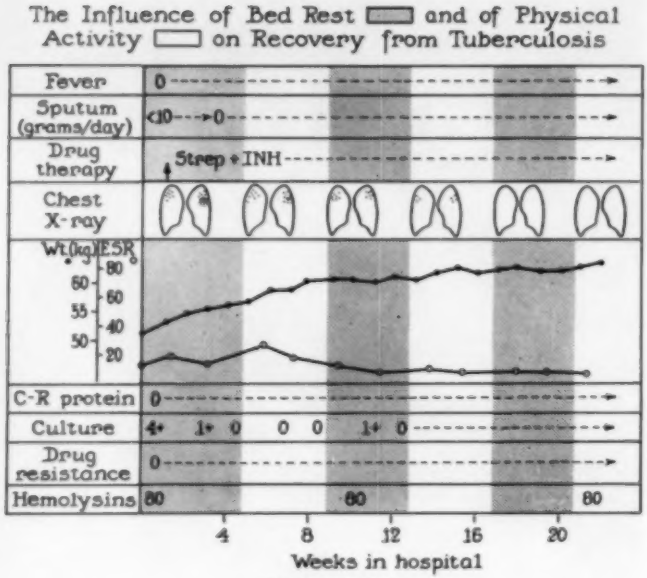
This patient's recovery was uncomplicated and, on September 2, 1955, seven months after admission, she was discharged from the hospital. Streptomycin was given for a period of one year, and isoniazid will be continued indefinitely. Regular follow-up examinations in the outpatient clinic reveal apparent arrest of the disease.



February 2, 1955

Case 2

August 8, 1955

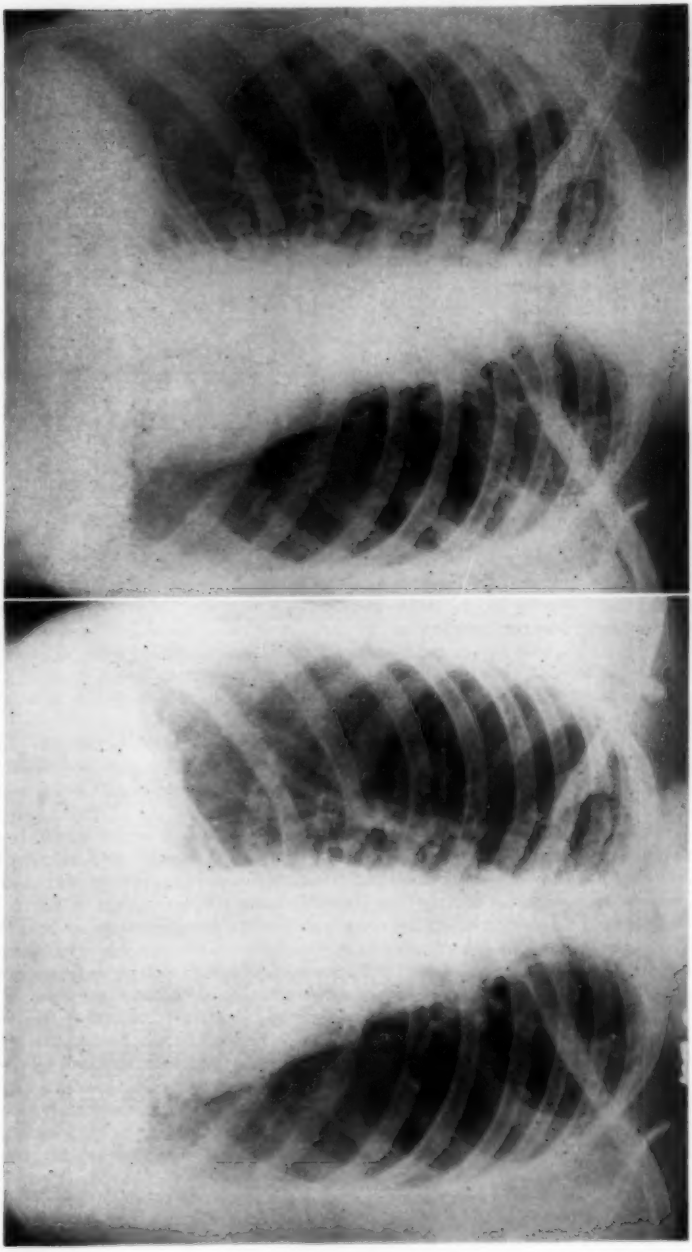


Case 3 (W. G.)

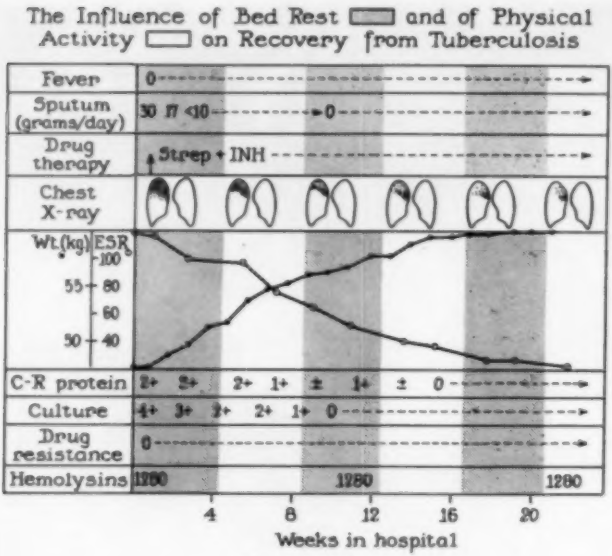
This patient, a 35-year-old married Negro female teacher, was admitted to the hospital on November 30, 1954. A chest roentgenogram taken during a pre-employment medical examination was reported to show probable tuberculosis. A chronic slightly productive cough had been present for four years. At the time of admission the patient also gave a history of night sweats, malaise, and vague aches in the chest. In 1939 a brother-in-law died of tuberculosis and in 1949 a close friend developed tuberculosis.

Physical examination revealed a well-nourished, well-developed Negro woman who did not appear ill. A small area of bronchovesicular breath sounds and rales was detected along the medial border of the left scapula.

The patient's course was uncomplicated and on May 20, 1955, after six months' hospitalization, she was discharged. Treatment consisted of a one-year course of streptomycin three times weekly and isoniazid, 5 mg. per kg. daily. The isoniazid is being continued indefinitely. Regular follow-up examinations have revealed no abnormalities.



December 13, 1954 Case 3 June 24, 1955



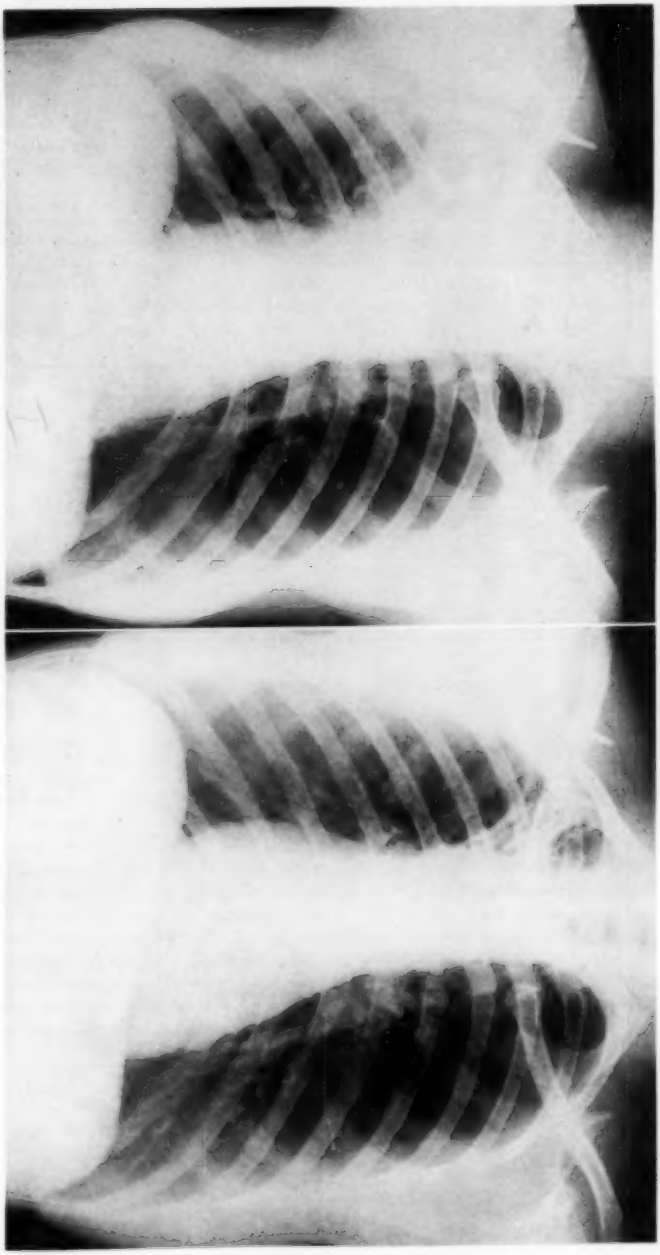
Case 4 (H. M.)

This patient, a 26-year-old Negro woman, was admitted to the hospital on December 3, 1954, six months post partum. During the three months before admission, she had a chronic productive cough, pain on the right side of the chest, occasional bloody sputum, and a 10 pound weight loss. In 1949 the patient worked in a tuberculosis sanatorium, but repeated chest roentgenograms, the last of which was obtained six months before admission, were said to be normal.

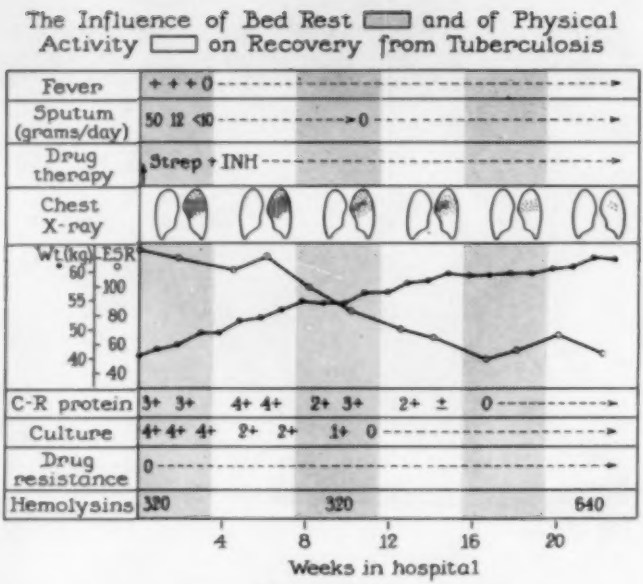
The patient was a small, underweight Negress who appeared chronically ill. The positive physical findings were limited to the chest where a lag on inspiration, increased fremitus, dullness on percussion, post-tussic rales, and bronchial breath sounds were detected over the right upper lobe.

Treatment consisted of streptomycin and isoniazid. Her recovery was uncomplicated and after six months' hospitalization she was discharged on June 10, 1955. Regular follow-up examinations in the outpatient clinic indicated continued improvement and after a period of one year, streptomycin therapy was stopped. Isoniazid was continued.

Despite medical advice to the contrary, this patient became pregnant, and in August, 1956, during the ninth month of pregnancy, findings were noted suggestive of reactivation of her tuberculosis. These included reappearance of cough and rales, roentgenographic changes of the residual shadows in the right apex, marked elevation in erythrocyte sedimentation rate over and above that observed during the pregnancy, and the detection of small numbers of acid-fast bacilli in a sputum smear. The patient was hospitalized in another institution where she delivered a normal male infant. Repeated sputum and gastric cultures revealed no tubercle bacilli. She signed out of the hospital against medical advice. On a clinic visit in December, 1956, the cough and rales had disappeared and the erythrocyte sedimentation rate had returned to nearly normal, but the roentgenographic picture was unchanged. All cultures are negative for *M. tuberculosis* to date. She is continuing to take isoniazid and, since she refuses hospitalization, will be followed as an outpatient. Her infant child is being cared for in another home until the patient's status is further clarified.



December 13, 1954 Case 4 May 15, 1955

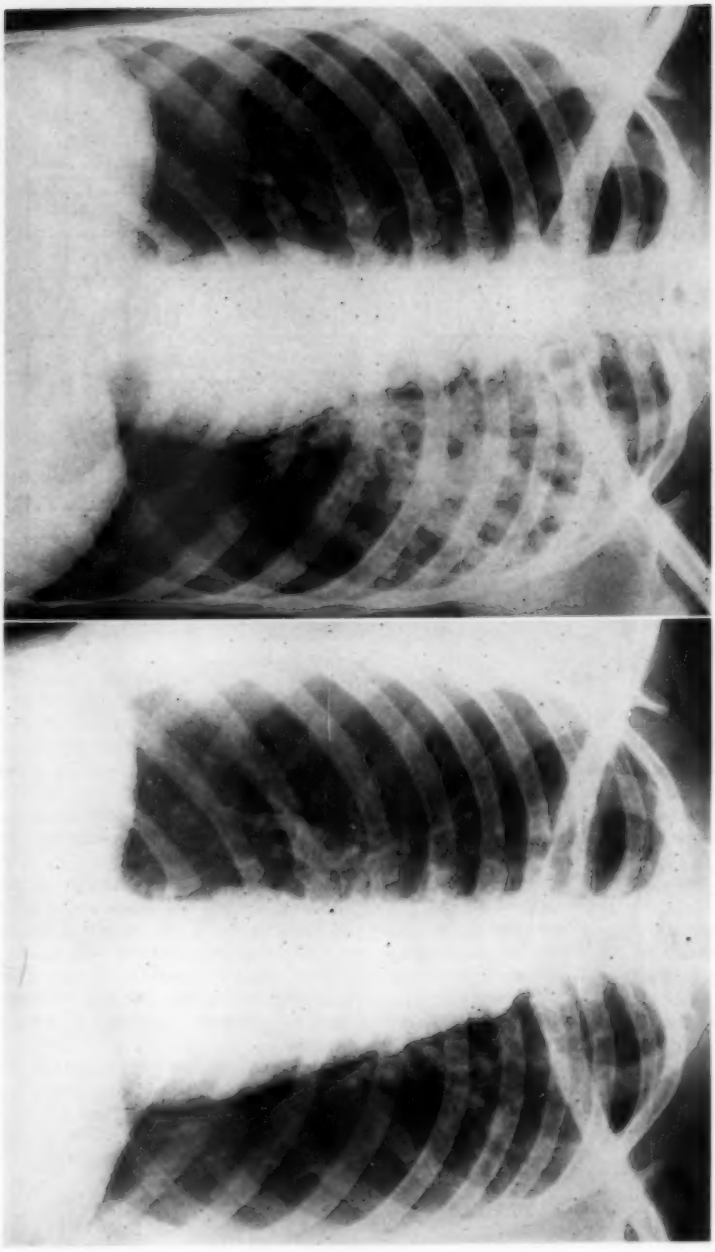


Case 5 (E. M.)

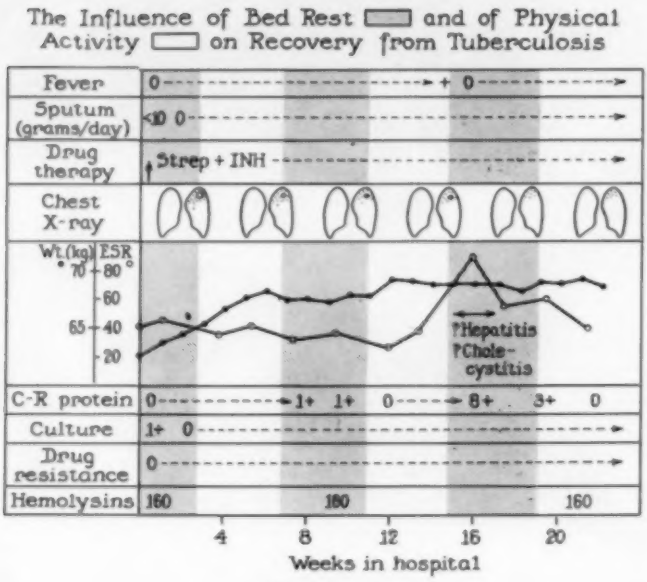
This patient, a 24-year-old divorced Negro female, was admitted to the hospital on December 9, 1954, with an illness of four months' duration characterized by an increasingly productive cough, pain over the left chest on deep respiration or coughing, a 25 pound weight loss, fever, night sweats, and dyspnea on exertion. A chest roentgenogram obtained in 1952 was said to be normal. There was no known exposure to tuberculosis.

Physical examination showed an acutely ill woman who was emaciated, toxic, and slightly dyspneic at rest. Over the distribution of the left upper lobe there was dullness on percussion, and harsh, medium, and fine rales were heard on auscultation.

Her progress was uncomplicated. Therefore, on July 15, 1955, seven months after admission, she was discharged from the hospital. Regular clinic visits indicated continued improvement. Streptomycin was administered for one year and isoniazid is being continued indefinitely. Despite medical advice to the contrary, she became pregnant and delivered a healthy son in April, 1956. No signs of relapse were noted during or following the pregnancy.



December 9, 1954 Case 5 May 31, 1955



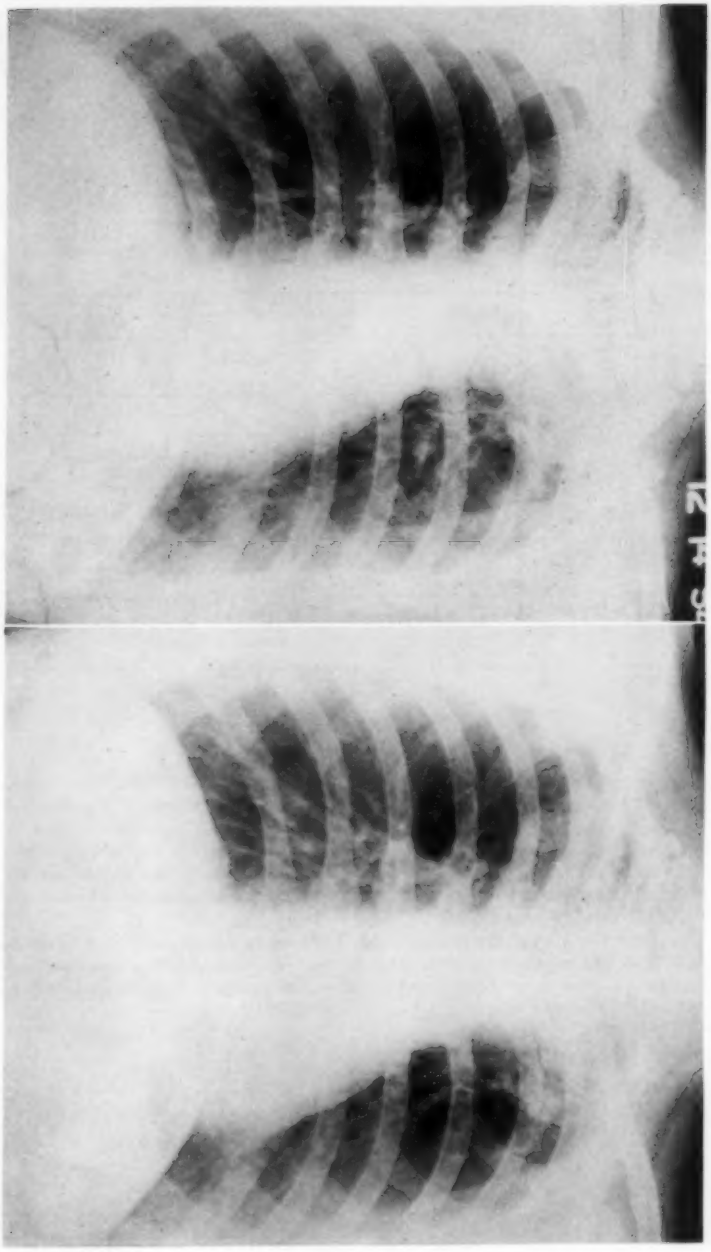
Case 6 (P. B.)

This patient, a 33-year-old white married woman, had known untreated active tuberculosis for four years when she was admitte l to the hospital on December 14, 1954. At that time she gave a history of chronic productive cough with several episodes of grossly bloody sputum, occasional left chest pain, and shortness of breath.

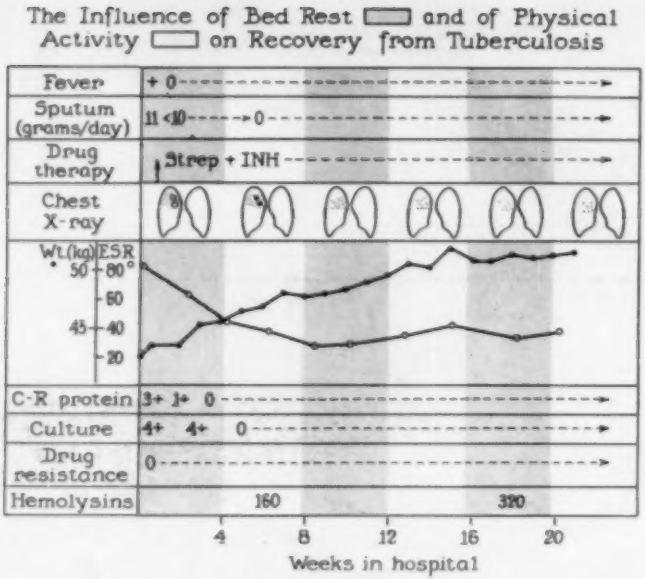
Physical examination revealed a well-developed, well-nourished white woman who did not appear ill. The significant positive findings were a limitation of respiratory movements on the left and rales over the entire left lung.

During March, 1955, the patient developed crampy right upper quadrant pains, watery stools, and pain in the right shoulder. The patient had slight tenderness in the right upper quadrant, but the liver was not palpable and examination of the chest was normal. Bilirubin, thymol turbidity, cholecystogram, urine and stool examinations were within normal limits. No definitive diagnosis was made. Within six days all symptoms abated. There were no other untoward events and the patient was discharged on July 15, 1955. Treatment consisted of streptomycin, 1 gm. three times a week for one year, and isoniazid, 5 mg. per kg. daily. The isoniazid therapy is still being continued.

During the sixteen months of follow-up observation in the clinic the patient has continued to do well. Immediately after discharge from the hospital she did her household chores and within six months returned to work in a jewelry manufacturing plant.



December 14, 1954 Case 6 June 13, 1955

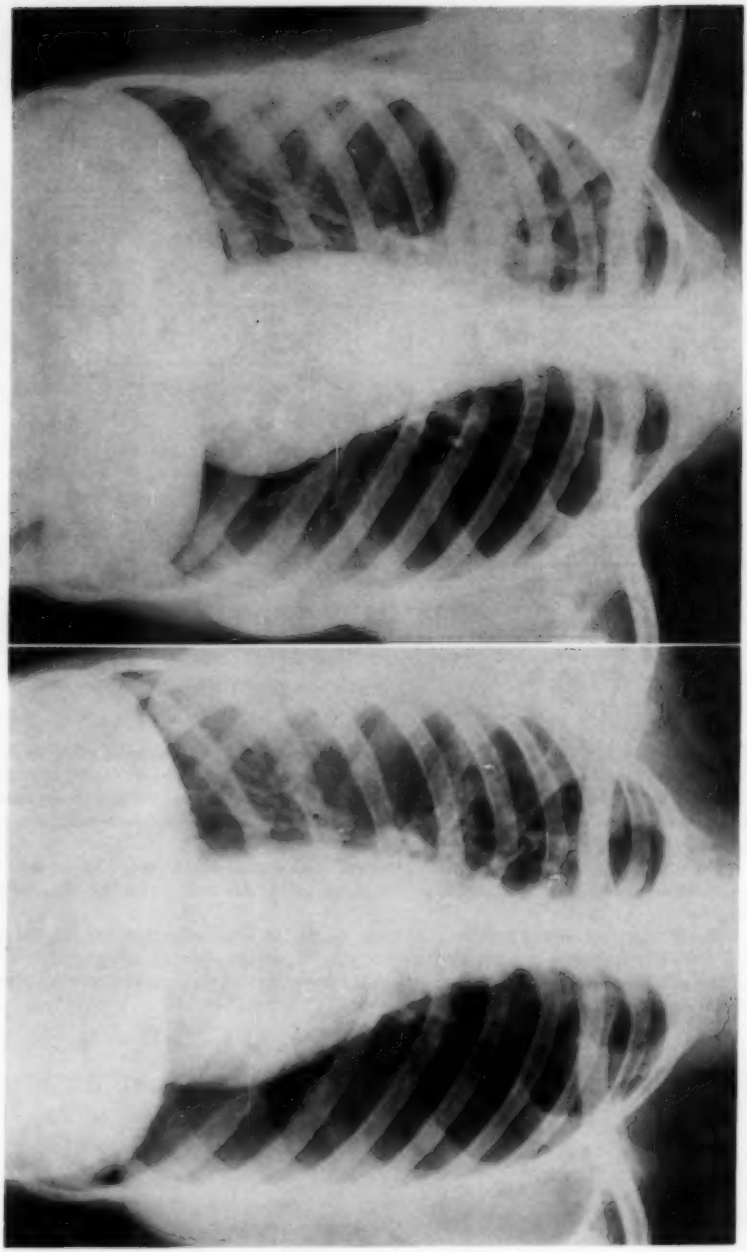


Case 7 (R. L.)

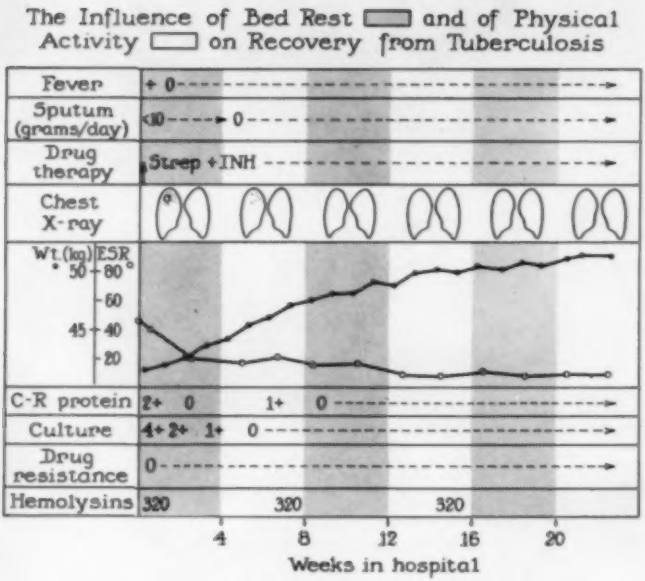
This patient, a 24-year-old married Negro woman, was admitted to the hospital on January 6, 1955, with an illness of two months' duration, characterized by a productive cough, occasional night sweats, fever, and a 10 pound weight loss. There was no recent or remote contact with tuberculosis. A chest roentgenogram taken in 1951 was said to be normal.

Physical examination revealed a thin, young, Negro woman who did not appear ill. The only significant positive findings were rales and increased transmission of voice sounds over the area of the right upper lobe.

The patient improved rapidly and after five months' hospitalization was discharged on June 3, 1955. She returned to work in December, 1955. She has been examined regularly in the outpatient clinic, and her disease appears to be arrested. Streptomycin was continued for one year. The isoniazid is being continued indefinitely.



January 6, 1955 Case 7 June 24, 1955

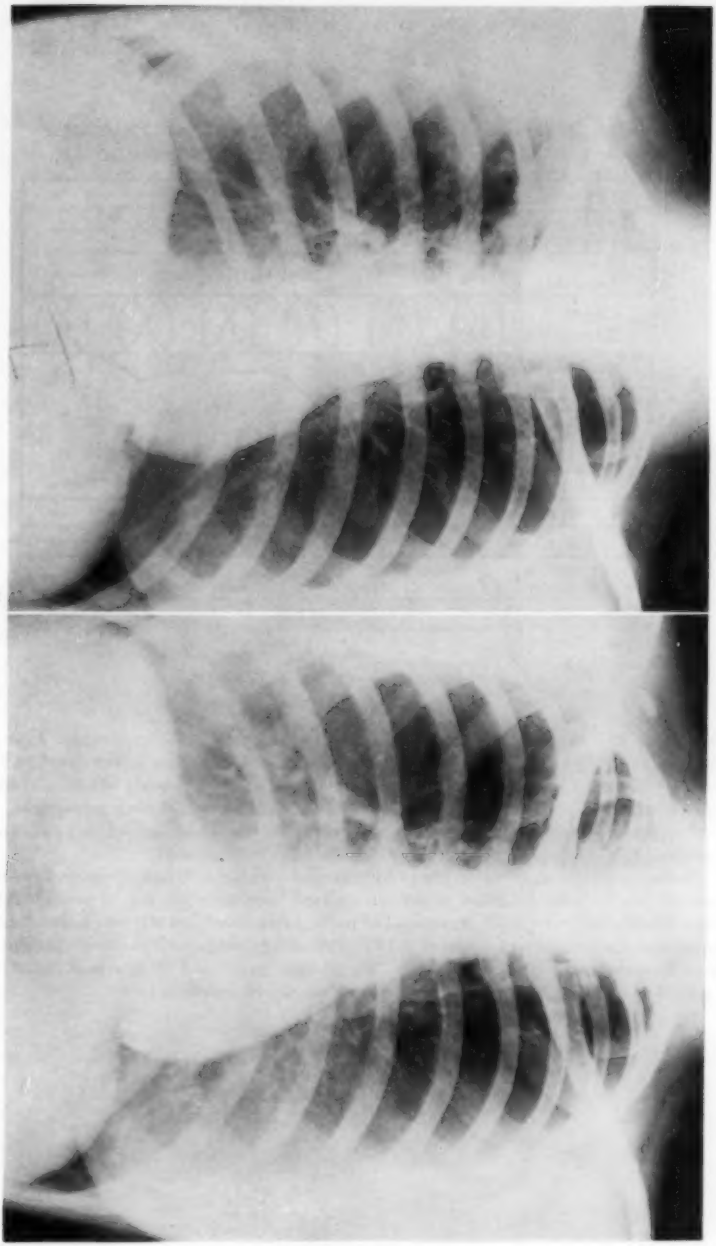


Case 8 (L. R.)

This patient, a 22-year-old married Puerto Rican woman, was admitted to the hospital on May 23, 1955, with an illness of five months' duration, characterized by chronic productive cough, a 15 pound weight loss, chest pain, nausea, and shortness of breath on exertion. Three weeks before admission, an 8-year-old nephew who was living in her home was hospitalized with tuberculosis.

The general appearance of the patient was that of a small woman who had recently lost a considerable amount of weight. On auscultation, rales were heard over the distribution of the right upper lobe.

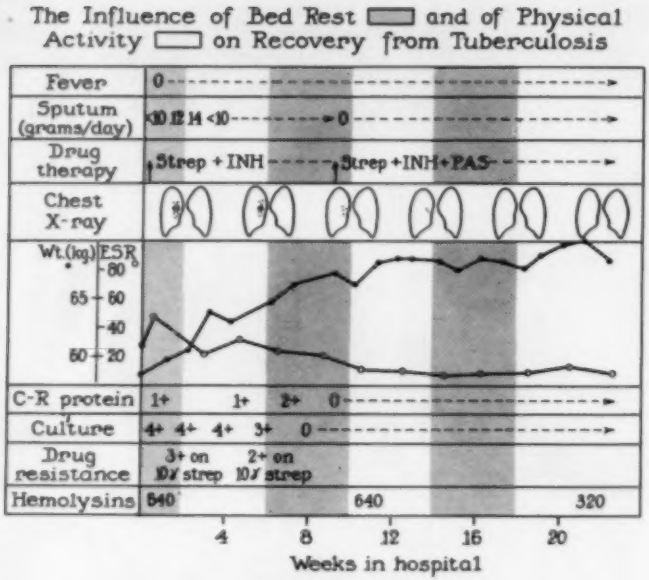
The patient responded well and after six months of hospitalization was discharged on December 7, 1955. Regular examinations in the outpatient clinic indicate apparent arrest of the disease. Streptomycin was continued for one year and isoniazid will be given indefinitely.



May 23, 1955

Case 8

November 14, 1955

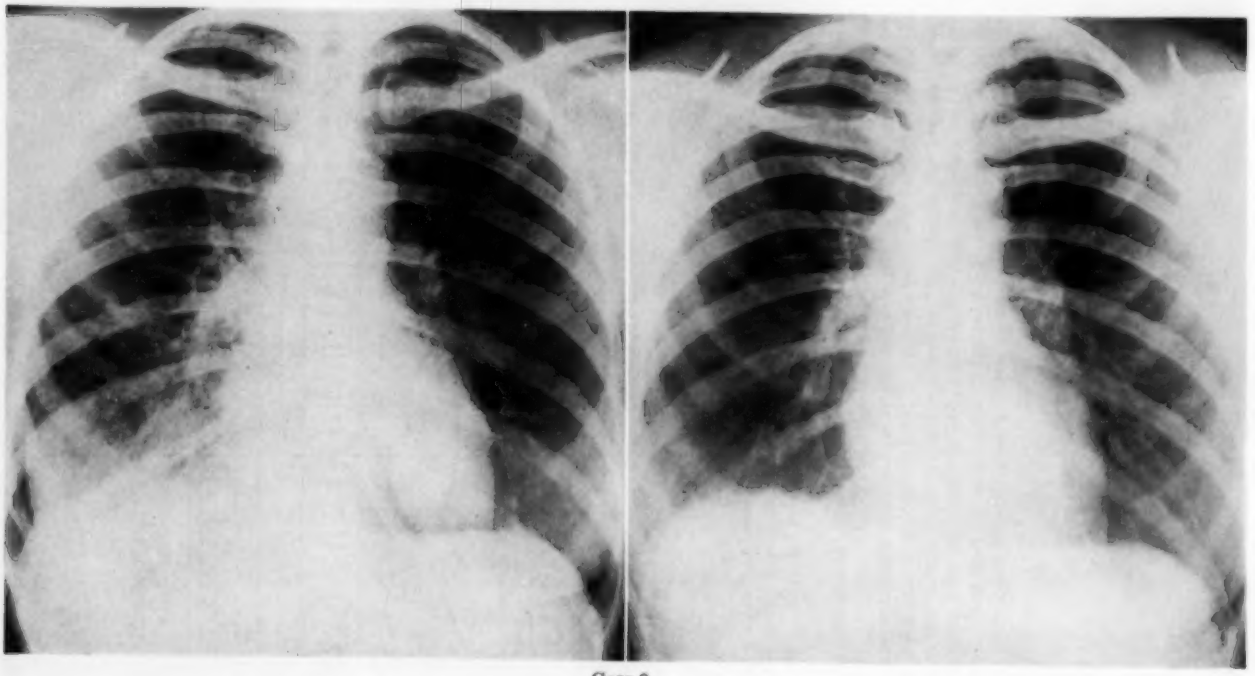


Case 9 (L. C.)

This patient, a 40-year-old Negro woman, was admitted to the hospital on June 6, 1955. Two months before admission she noted the onset of a chronic productive cough, a 10 pound weight loss, fever, occasional night sweats, malaise, and one episode of hemoptysis. There was no history of contact with tuberculosis nor of previous chest roentgenograms.

She appeared well developed and slightly toxic. At the tip of the right scapula an area of bronchial breath sounds with a few post-tussic rales were present.

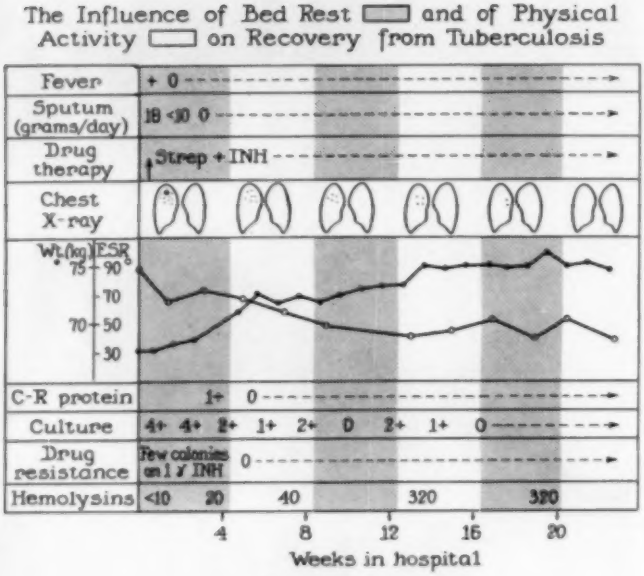
Chemotherapy consisted of streptomycin and isoniazid. When streptomycin-resistant tubercle bacilli were detected in several sputum specimens, 15 gm. of sodium PAS daily was added to the therapeutic regimen. The patient improved and after six months' hospitalization was discharged on December 2, 1955. Periodic examinations in the outpatient department have revealed no abnormalities. The streptomycin and PAS were administered for a period of one year and the isoniazid will be continued indefinitely.



Case 9

June 6, 1955

November 28, 1955



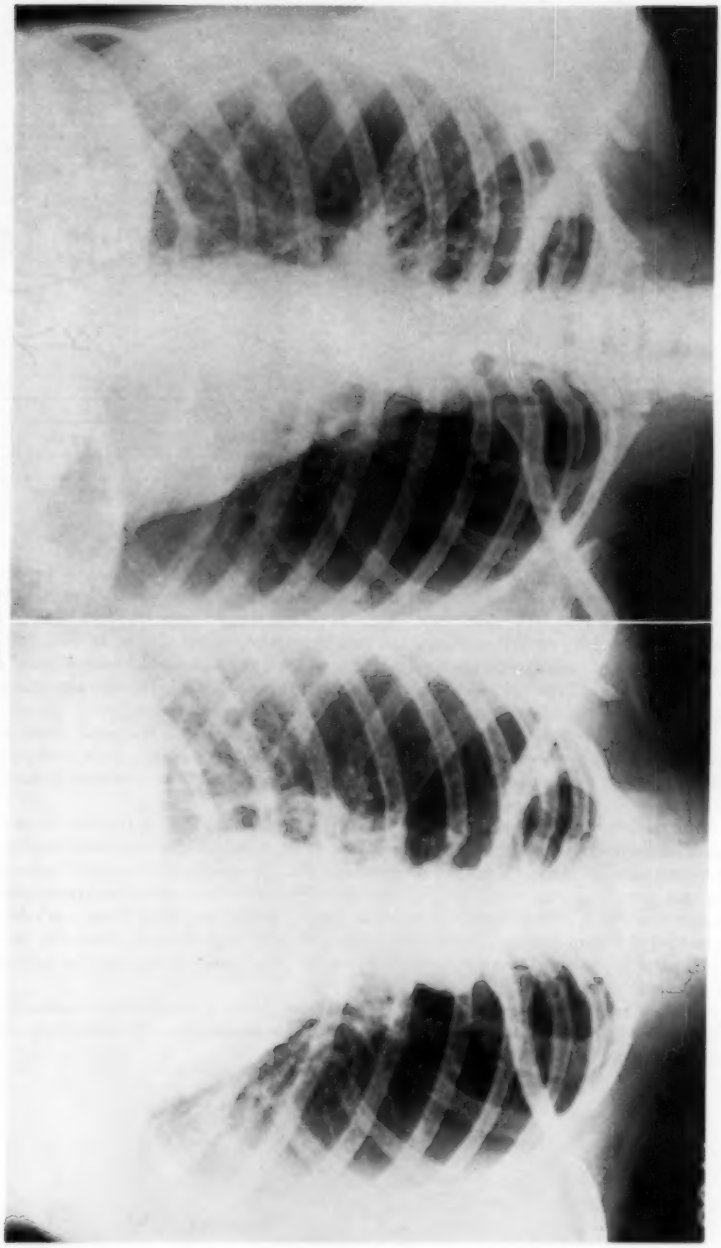
Case 10 (V. B.)

This patient, a 35-year-old divorced Negro woman, was admitted to the hospital on June 10, 1955. She became hoarse with a cough productive of small amounts of blood-tinged sputum about three months before admission. A roentgenogram taken at her place of employment revealed infiltration and cavitation.

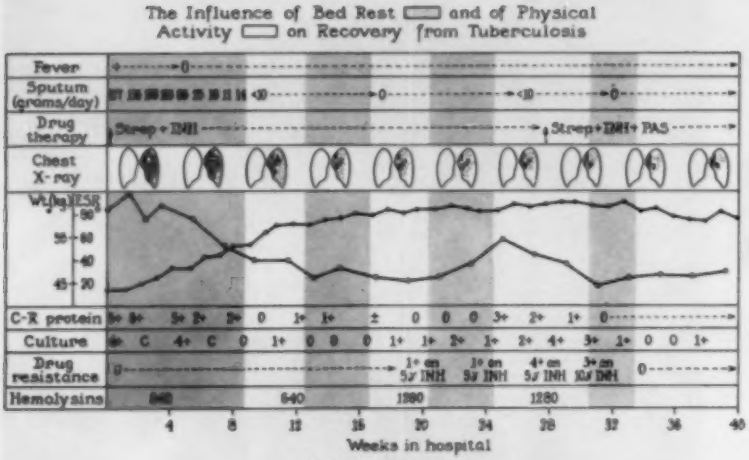
This patient was well developed and slightly overweight. Harsh rhonchi, fine and medium rales were heard over the upper one-third of the right chest.

In early cultures of the sputum a slight degree of isoniazid resistance was detected. Therapy was not changed since the numbers of isoniazid-resistant organisms which appeared were few, and subsequent sputum examinations revealed no resistant organisms.

The patient responded well, and was discharged on December 19, 1955, after six months' hospitalization. By April, 1956, she returned to work on a full-time basis. Streptomycin was continued for one year and isoniazid is being administered indefinitely. Follow-up examinations have revealed no abnormal findings.



June 27, 1955 Case 10 November 14, 1955



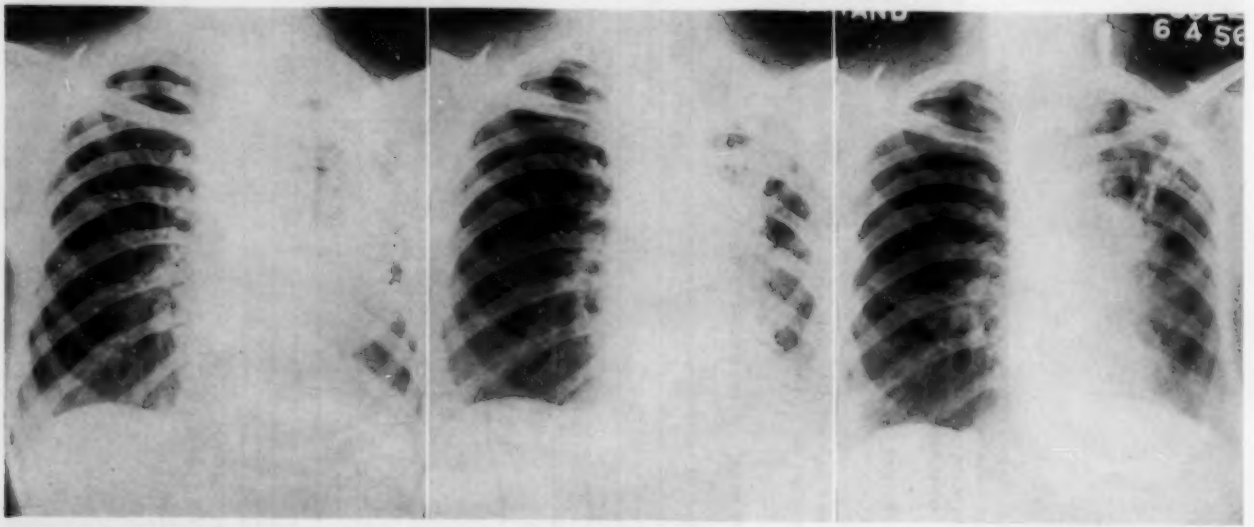
Case 11 (M. B.)

This patient, a 22-year-old Negro woman, was admitted to the hospital on September 6, 1955, with an illness of two months' duration characterized by productive cough, fever, night sweats, severe left chest pain, and a 20 pound weight loss. She had been in a penal institution for drug addiction during the two years prior to her illness. In 1952, the patient was hospitalized for bilateral cervical adenitis which was diagnosed as tuberculous. A roentgenogram at this time did not reveal pulmonary disease. No specific therapy was given. A brother and sister have tuberculosis and are receiving chemotherapy.

The patient was debilitated and extremely ill on admission. Bilateral, hard, enlarged cervical nodes were present. Respiratory movements were markedly decreased on the left. The entire left chest was dull to percussion. Marked diminution of normal breath sounds and numerous harsh rales and rhonchi were present.

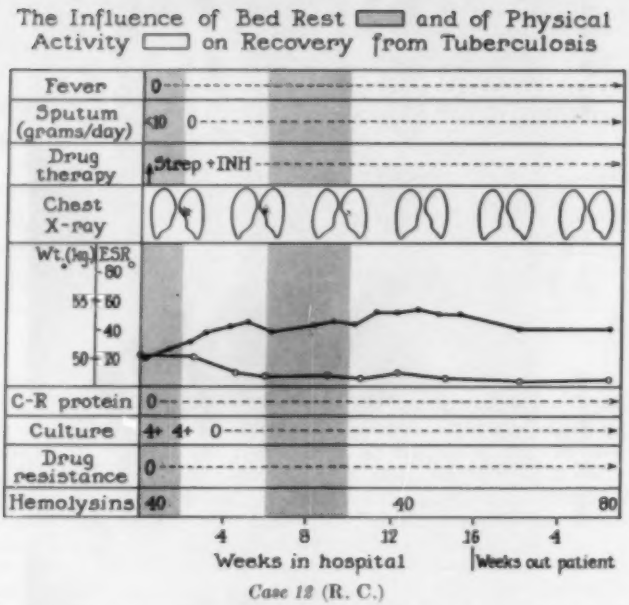
The patient was treated with isoniazid and streptomycin. During the first three weeks of hospitalization her daily temperature reached at least 40° C. She improved gradually until the sixth month of hospitalization when tubercle bacilli resistant to isoniazid were detected. At this time physical examination, laboratory studies, and chest roentgenograms were suggestive of possible relapse. Sustained-action PAS in 12 gm. daily doses was added to the isoniazid and streptomycin therapy. She again showed improvement; however, on June 15, 1956, after nine months' hospitalization, she left the hospital against the advice of her physicians.

She has been seen irregularly in the outpatient clinic. Examinations conducted during these visits have not shown any deterioration to the present time. Streptomycin, isoniazid, and PAS have been continued to date.



Case 11

September 6, 1955 February 14, 1956 June 4, 1956

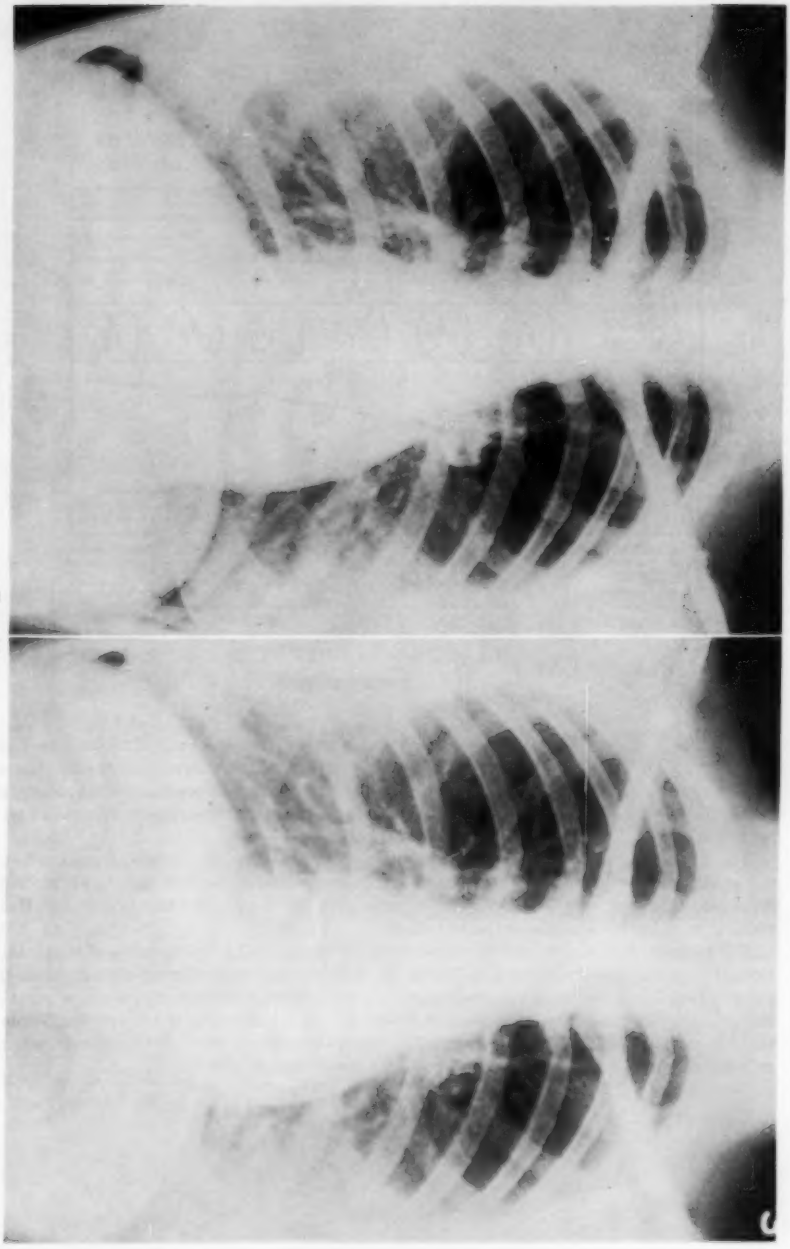


Case 12 (R. C.)

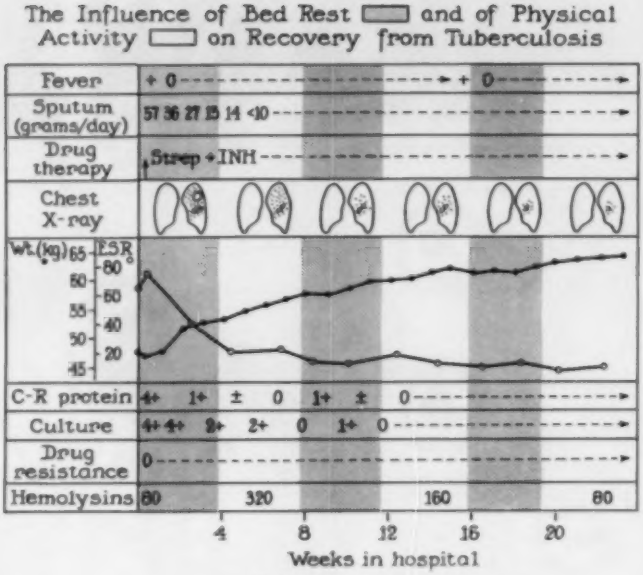
This patient, an 18-year-old Negro girl, was admitted to the hospital on September 26, 1955. A roentgenogram taken at her place of employment revealed a cavity in the left hilar area. Her only complaint at this time was a chronic nonproductive cough. A roentgenogram taken in June, 1955, was normal. There was no history of contact with tuberculosis.

Physical examination revealed a well-developed, healthy-looking young girl, with no significant positive findings.

The patient responded rapidly and she was discharged on January 13, 1956, after four months' hospitalization. By March, 1956, she was back at work and leading an active life. At present her chest roentgenogram is normal, as are all laboratory studies. Streptomycin was stopped after a period of one year, but isoniazid will be continued indefinitely.



September 26, 1955 Case 12 March 2, 1956

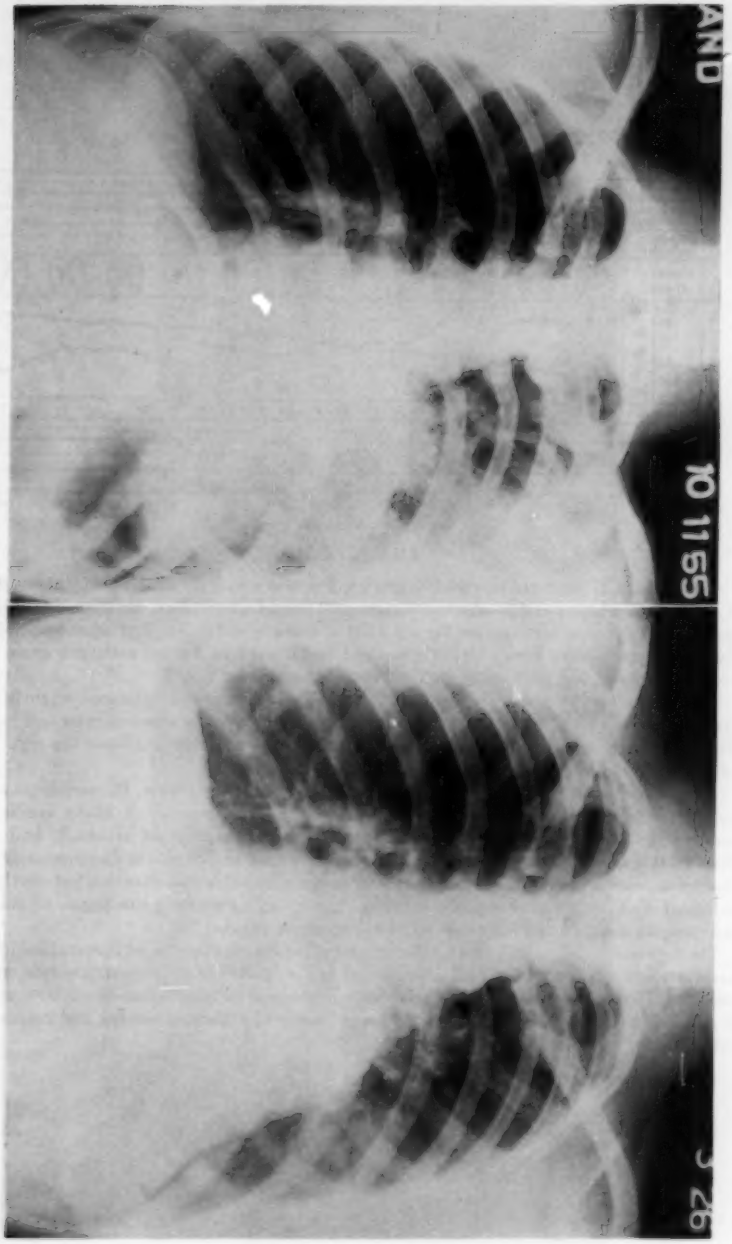


Case 13 (K. H.)

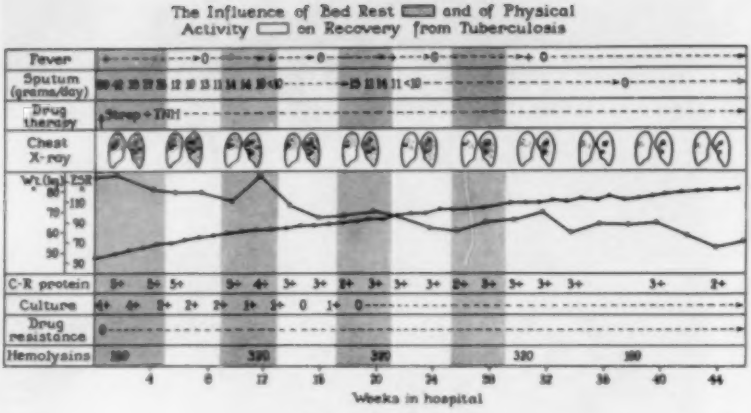
This patient, a 13-year-old Negro school girl, was admitted on October 11, 1955, with an illness of four weeks' duration characterized by productive cough, left anterior chest pain, hoarseness, anorexia, night sweats, and malaise. There was no history of previous chest roentgenograms nor was there any known contact with tuberculosis. Menarche was at the age of twelve years.

The patient was well developed and looked older than her stated age. Examination of the chest revealed limitation of respiratory movements on the left side, with dullness, decreased fremitus, and fine and medium rales over the area of the left lower lobe. Harsh rhonchi were heard throughout the left chest.

Her progress was steady and uncomplicated. After five and a half months of hospitalization, the patient was discharged on March 29, 1956. On regular follow-up examinations this patient has shown continued improvement. At the present time, her laboratory studies are normal and the chest roentgenogram shows progressive hardening of the residual focus in the left midlung field. In September, 1956, she returned to school. Streptomycin was administered for one year and isoniazid will be continued indefinitely.



October 11, 1955 Case 13 March 26, 1956



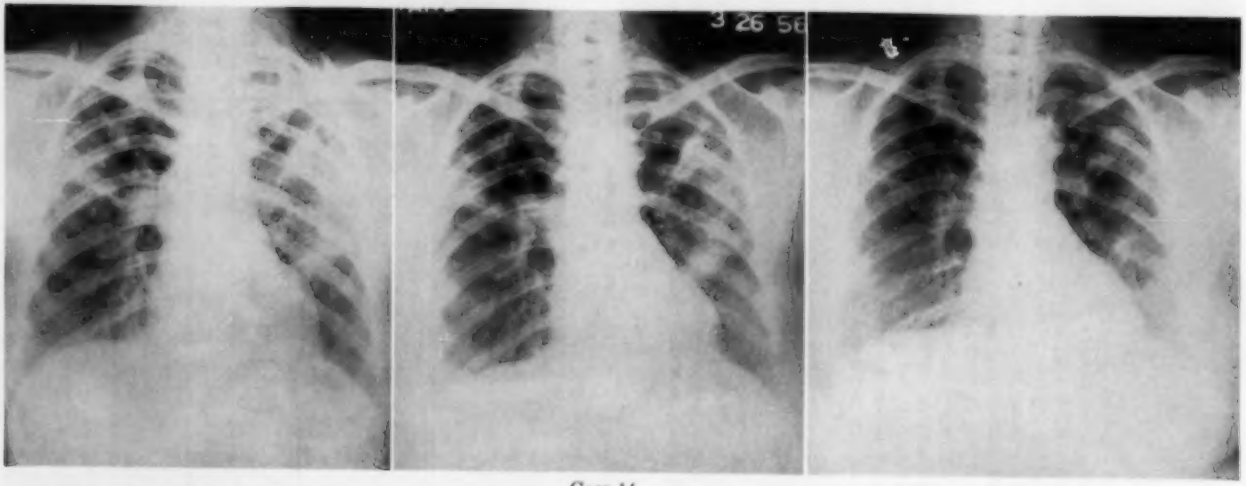
Case 14 (J. Mo.)

This patient, a 37-year-old married Negro woman, was admitted to the hospital on October 31, 1955, with a history of productive cough, a 20 pound weight loss, occasional fever, night sweats, and dyspnea on exertion for a period of three months. She had amenorrhea during the two years prior to admission. The patient had never had a chest roentgenogram previously and there was no known contact with tuberculosis.

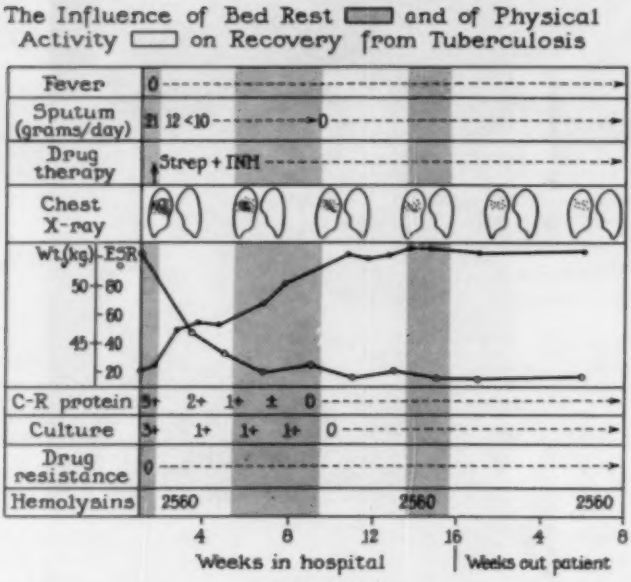
She was a short, stocky, well-nourished, Negro woman who appeared slightly toxic. Breath sounds were diminished throughout the left chest with occasional rales and rhonchi. Rhonchi were heard over the entire right chest and, over the distribution of the right upper lobe, cogwheel breath sounds were detected.

As is shown in the chart, her response to therapy was quite slow. Bacteriologic studies failed to reveal any tubercle bacilli resistant to the drugs employed. Many studies were conducted to eliminate the possibility of sarcoidosis, rheumatoid arthritis, and fungal disease. It was finally concluded that this patient's slow response was due primarily to the enormous amount of pulmonary disease. After six months' hospitalization her erythrocyte sedimentation rate had declined somewhat, the chest roentgenogram began to show improvement, and menstruation recurred and became regular.

On September 28, 1956, she was discharged after eleven months of hospitalization. Her roentgenogram revealed residual disease and her erythrocyte sedimentation rate was still elevated. However, these abnormalities have continued to improve on home treatment. It is planned to administer streptomycin for a period of eighteen months and isoniazid indefinitely.



November 14, 1955 | Case 14 March 26, 1956 | August 13, 1956

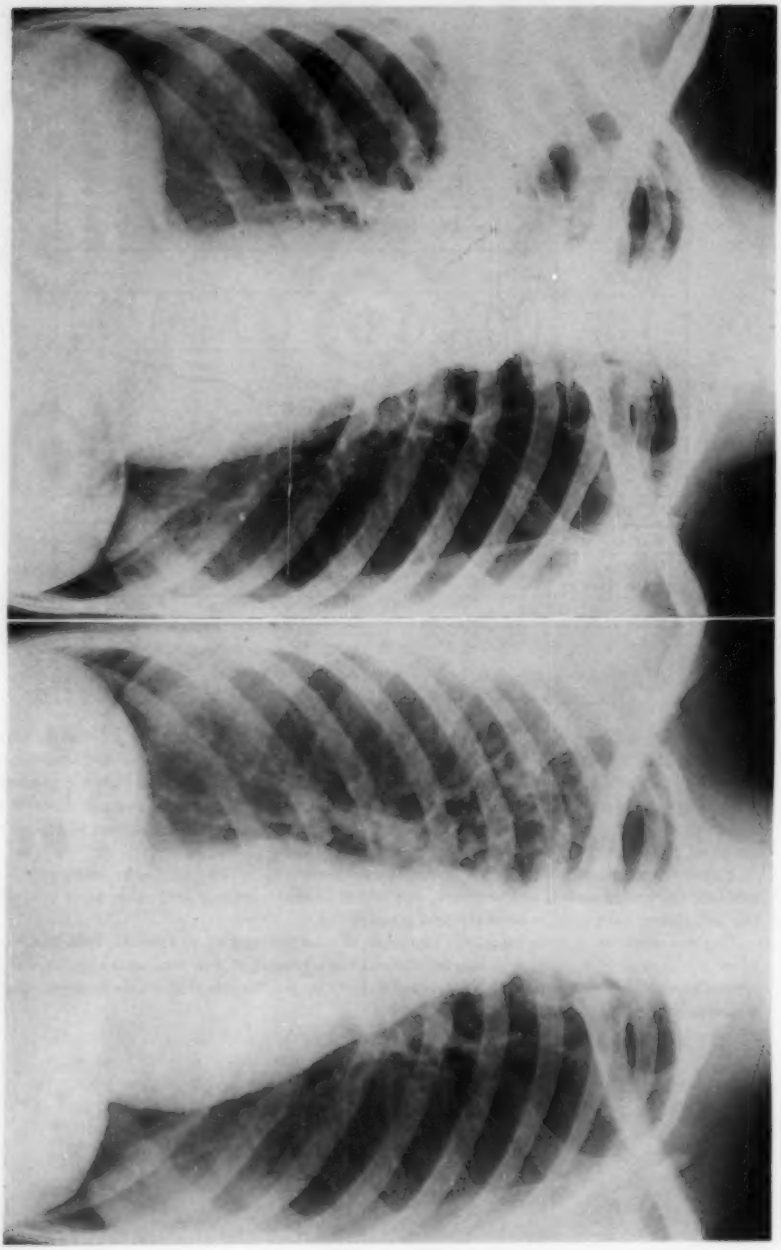


Case 15 (J. Ma.)

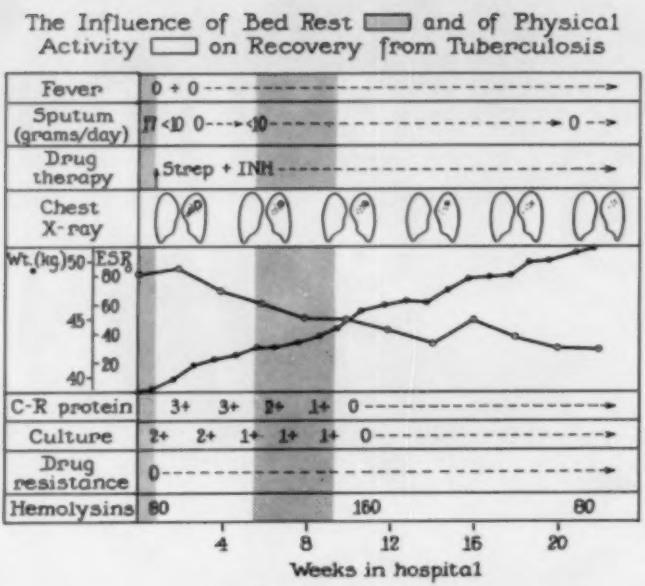
This patient, a 32-year-old Negro married woman, was admitted to the hospital on November 11, 1955, with an illness of four weeks' duration characterized by malaise, anorexia, fever, aching right chest pain, and a cough productive of non-bloody sputum. Annual chest roentgenograms, the last of which was taken a year before admission, were said to be normal. There was no history of recent or remote exposure to tuberculosis.

The patient was a thin Negro woman who appeared chronically ill. Over the area of the right upper lobe there were dullness to percussion, harsh bronchovesicular breath sounds, and many fine post-tussic rales.

Chemotherapy consisted of isoniazid and streptomycin. The patient improved rapidly and by the beginning of February, 1956, her sputum was free of acid-fast bacilli, the erythrocyte sedimentation rate was normal, and no evidence of cavitation could be detected in standard chest roentgenograms. However, on February 20, 1956, after approximately four months of hospitalization, she left the hospital against the advice of her physicians. She was seen at frequent intervals in the outpatient clinic. At first it appeared that she was continuing to improve while at home, but in August, 1956, weight loss, elevated erythrocyte sedimentation rate, reappearance of rales, and cavitation demonstrable by roentgenography were noted. Upon questioning the patient admitted that for the previous two months she had not taken any of her streptomycin or isoniazid. Rehospitalization was advised, but the patient decided to place herself under the care of a private physician to be treated at home. Efforts to obtain further follow-up data have not been successful.



November 11, 1955 Case 15 May 25, 1956

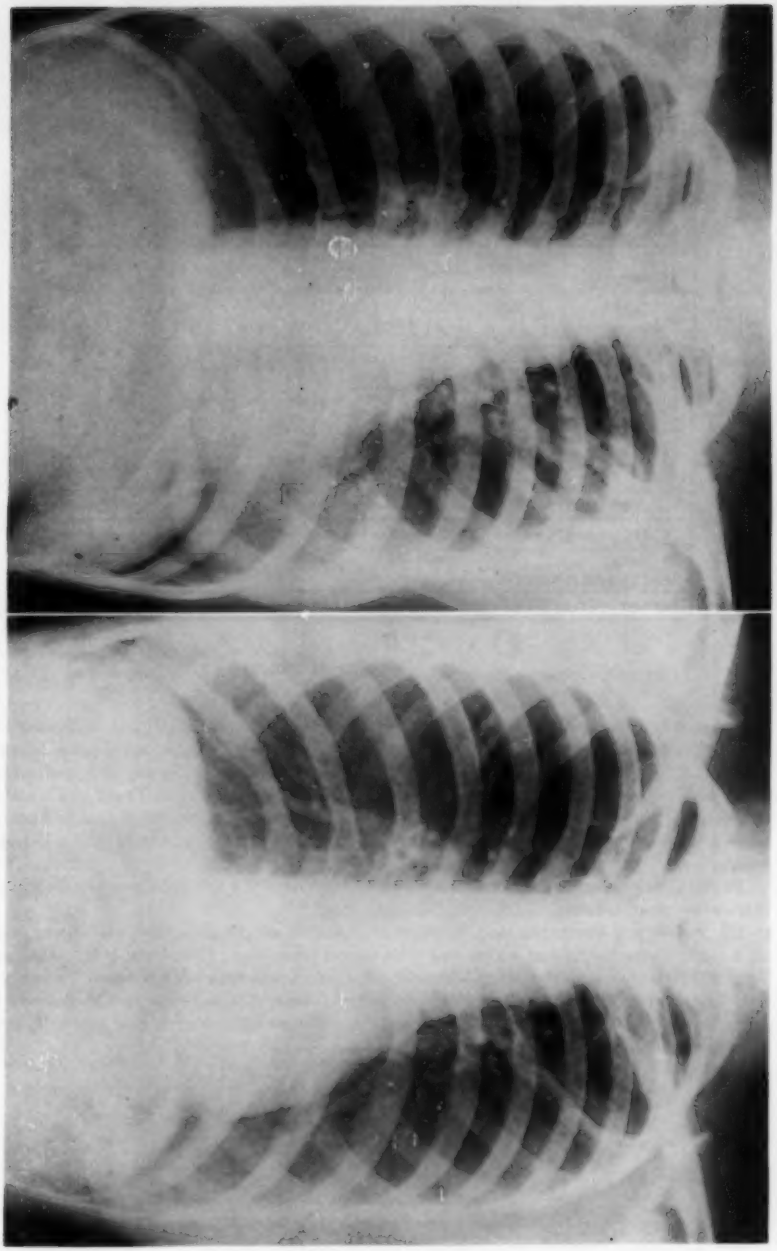


Case 16 (G. G.)

This patient, a 33-year-old Negro woman, who was separated from her husband, was admitted to the hospital on January 6, 1956, with an illness of four months' duration characterized by fatigue, malaise, a 10 pound weight loss, loss of appetite, and chronic productive cough. At the time of admission she also complained of fever and night sweats. A sister with whom she was living developed tuberculosis in 1949. A chest roentgenogram of the patient taken at that time was normal.

Physical examination at the time of admission revealed a thin, small, hyperkinetic Negro woman. On examination of the chest, diminished breath sounds and rales were heard over the left upper lung field anteriorly and posteriorly.

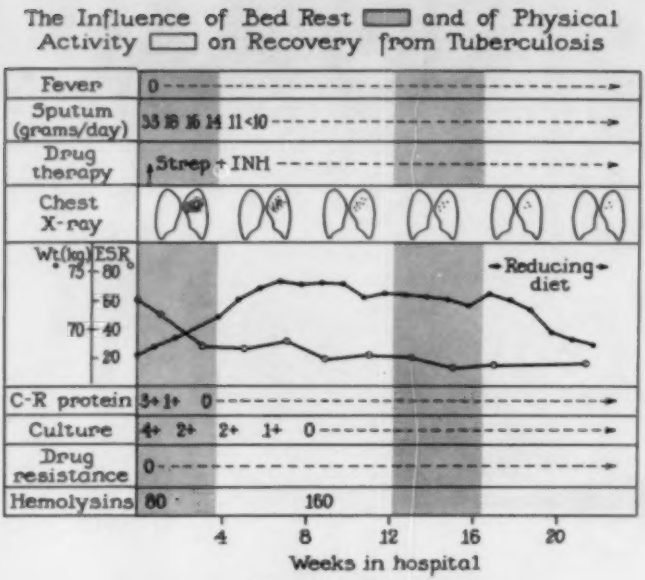
Her recovery was uncomplicated. The patient was discharged on June 27, 1956, six months after admission. Streptomycin was continued for a period of one year and isoniazid will be given for an indefinite period of time. She has been seen at regular intervals in the outpatient clinic and continues to do well.



January 6, 1956

Case 16

June 4, 1956

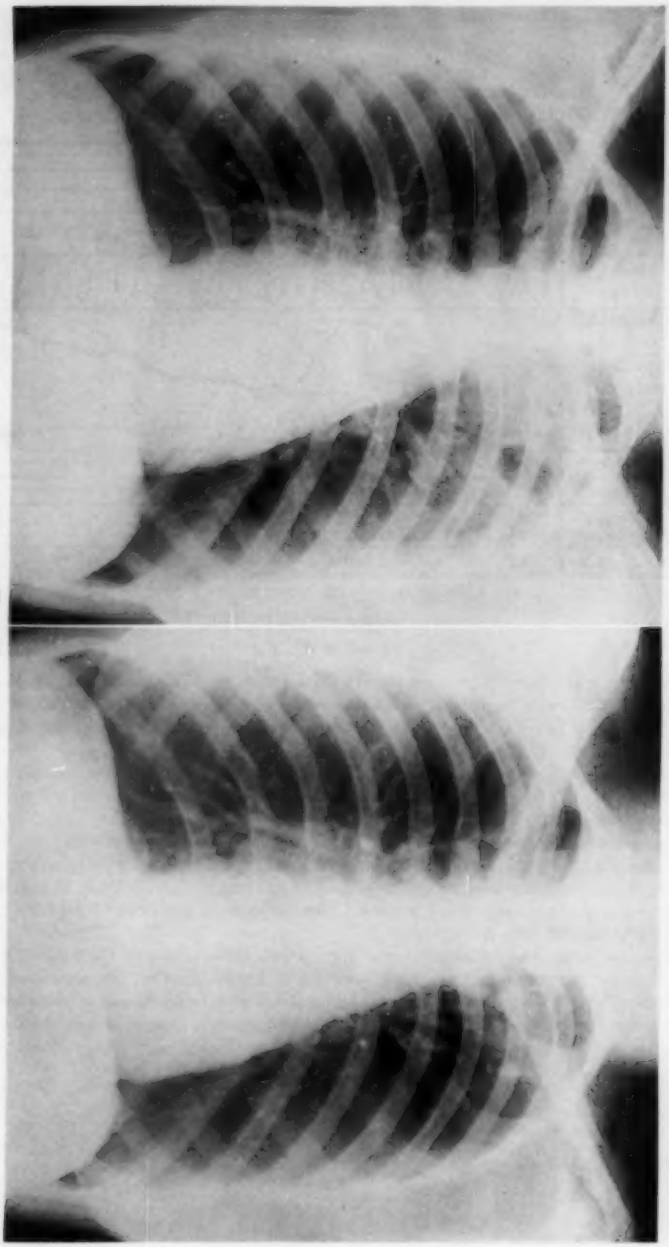


Case 17 (E. T.)

This patient, a 19-year-old Negro housewife, was admitted to the hospital on January 25, 1956. An infiltration in the lung was detected in a routine chest roentgenogram taken in July, 1955. Repeated roentgenographic examinations verified the lesion, and sputum cultures were reported as positive for tubercle bacilli in December, 1955. There was a history of a chronic cough since July, 1955, but in the latter part of December the illness had become more acute with severe cough, fever, night sweats, left posterior chest pain, and a 15 pound weight loss.

Physical examination on admission revealed rales over the left apex posteriorly but was otherwise unremarkable.

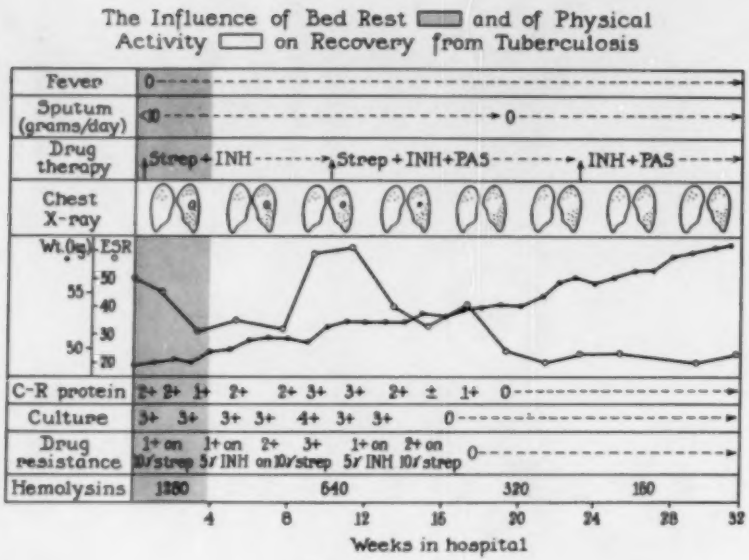
Her course was uncomplicated. Because of excessive weight gain, the patient was placed on a moderate reducing diet which was maintained from May, 1956, until her discharge on June 27, 1956, five months after admission. Streptomycin was administered for one year and isoniazid will be continued indefinitely. On follow-up examinations the patient has been asymptomatic, with a normal erythrocyte sedimentation rate and stable fibrocalcific densities in the left apex.



January 25, 1956

Case 17

July 27, 1956



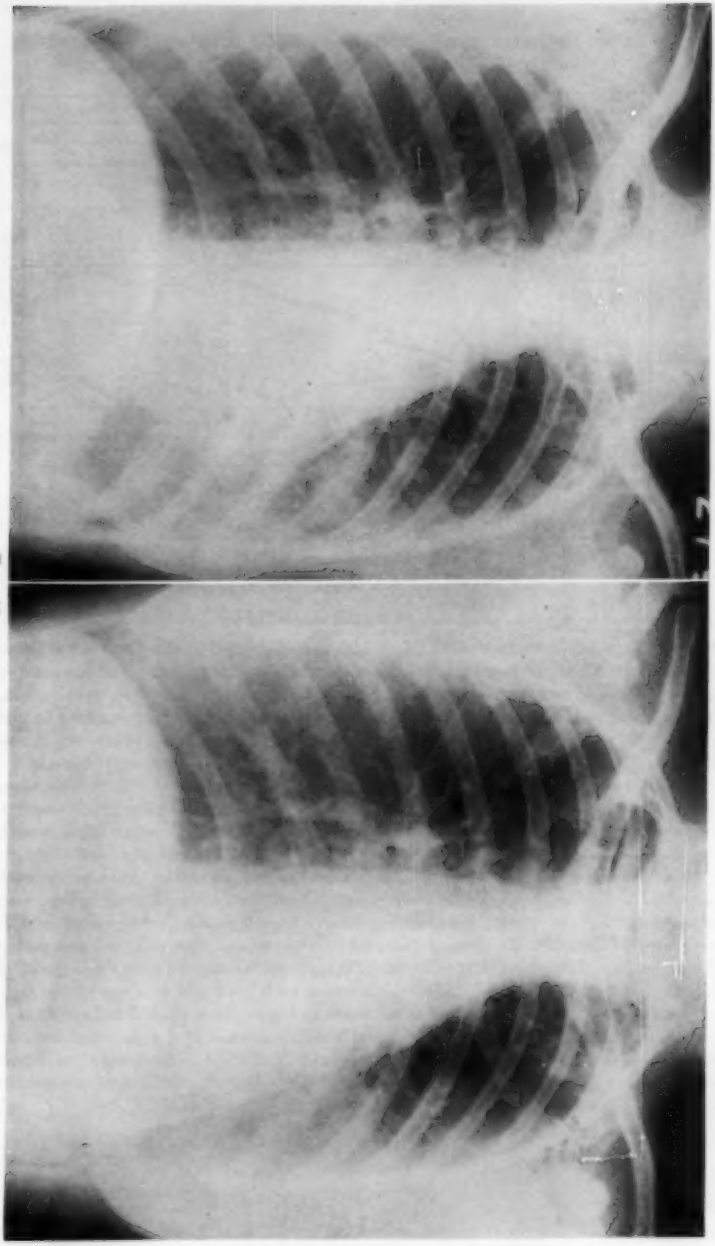
Case 18 (C. T.)

This patient, a 21-year-old married Puerto Rican woman, was admitted to the hospital on February 7, 1956, with an illness characterized by chronic productive cough, fever, chills, night sweats, left lower chest pain, and a 15 pound weight loss, all of four months' duration. She had experienced three episodes of slight hemoptysis.

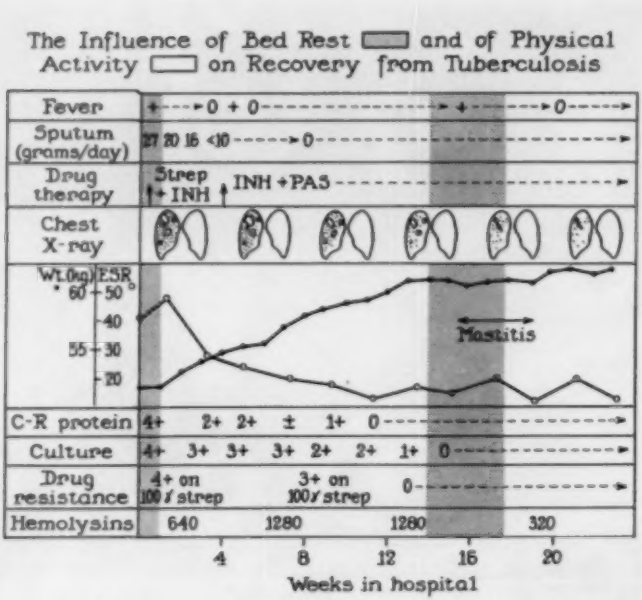
The patient appeared well developed and well nourished. On auscultation of the chest, a few crepitant rales were detected at the tip of the left scapula, and breath sounds were harsh and prolonged over the area of the left lower lobe.

Original treatment consisted of streptomycin and isoniazid. However, when tubercle bacilli resistant to streptomycin and isoniazid were repeatedly cultured from this patient's sputum, including the specimen obtained prior to therapy, sodium PAS, 15 gm. daily, was added to the chemotherapeutic regimen. Later, in view of persistent streptomycin resistance, streptomycin was discontinued.

Coincident with the administration of appropriate chemotherapy, the patient's improvement became accelerated. After eight months of hospitalization she was discharged on September 28, 1956. Since her discharge she has been able to perform her household duties, and regular clinic visits indicate continued improvement. Isoniazid and PAS have been continued to date.



February 7, 1956 Case 18 August 27, 1956

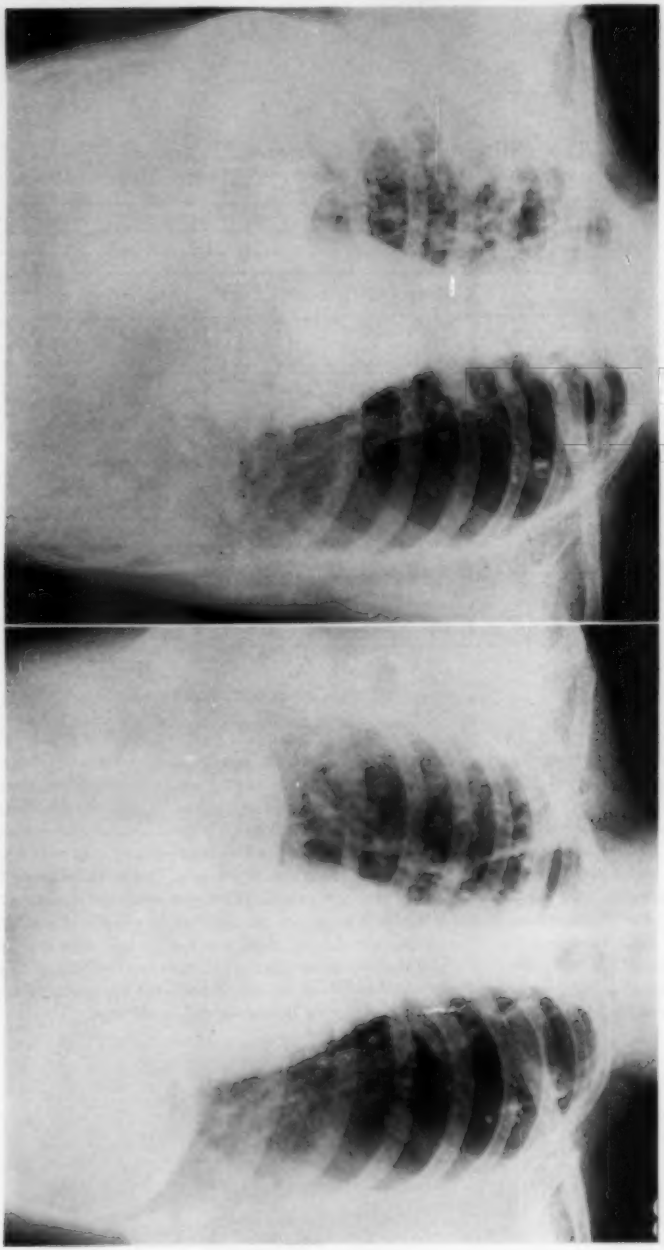


Case 19 (M. V.)

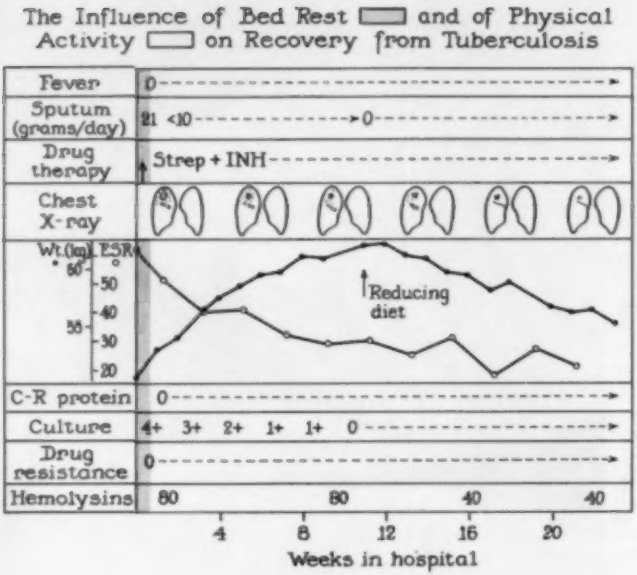
This patient, a 19-year-old Puerto Rican girl, was admitted to the hospital on February 21, 1956, with an illness of five months' duration characterized by chronic productive cough, loss of appetite, right anterior chest pain with coughing and deep respiration, fever, night sweats, and a 15 pound weight loss. The patient's mother succumbed to pulmonary tuberculosis when the patient was two years of age. She had had yearly chest roentgenograms, the last of which, three years prior to admission, was said to be normal.

On physical examination, the patient appeared well nourished and well developed. The entire right chest was less resonant than the left. Vocal fremitus was greater over the right lung and fine, medium, and coarse rales were heard over the right upper lobe. Breath sounds over the right middle and right lower lobes were diminished and fine rales were present.

Treatment consisted of streptomycin, 1 gm. intramuscularly three times weekly, and daily isoniazid, 5 mg. per kg. of body weight. However, approximately three weeks after admission, tubercle bacilli markedly resistant to streptomycin were cultured from the pretreatment sputum. In view of this, streptomycin was discontinued and sodium PAS, 15 gm. per day, was added to the therapeutic regimen. Three and a half months after admission the patient developed a unilateral mastitis with pain and swelling of the right breast and elevation of temperature. All acute signs and symptoms abated in four days, but low-grade fever and recurrent discomfort in the breast continued for approximately four weeks. Her hospitalization was otherwise uncomplicated and she was discharged on September 20, 1956, seven months after admission. Return visits to the clinic indicate continued improvement. Isoniazid and PAS have been continued to date.



February 27, 1956 Case 19 August 27, 1956

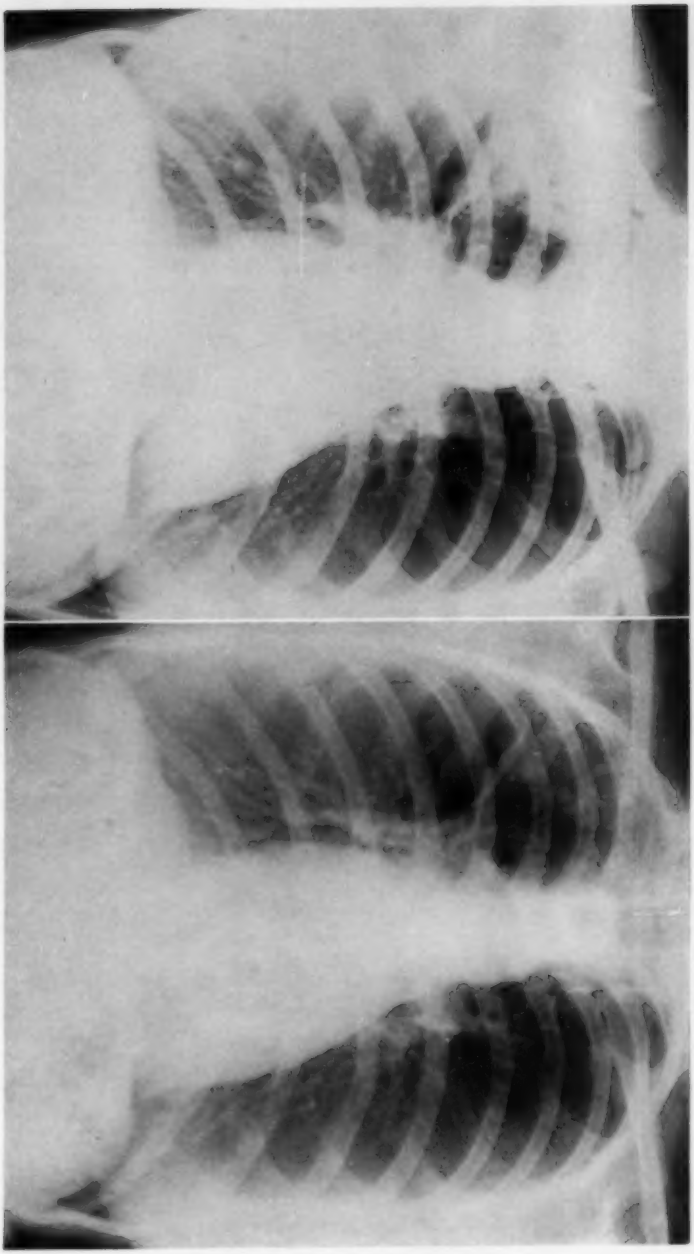


Case 20 (B. B.)

This patient, a 30-year-old Negro woman, separated from her husband, was admitted to the hospital on March 7, 1956, with an illness of one month's duration characterized by cough productive of sputum which was occasionally blood tinged. No previous chest roentgenograms were ever taken and there was no known contact with tuberculosis.

On physical examination, the patient appeared well developed and well nourished, with no evidence of weight loss. Slight dullness and a few post-tussic rales were detected over the right apex posteriorly; otherwise the examination was essentially normal.

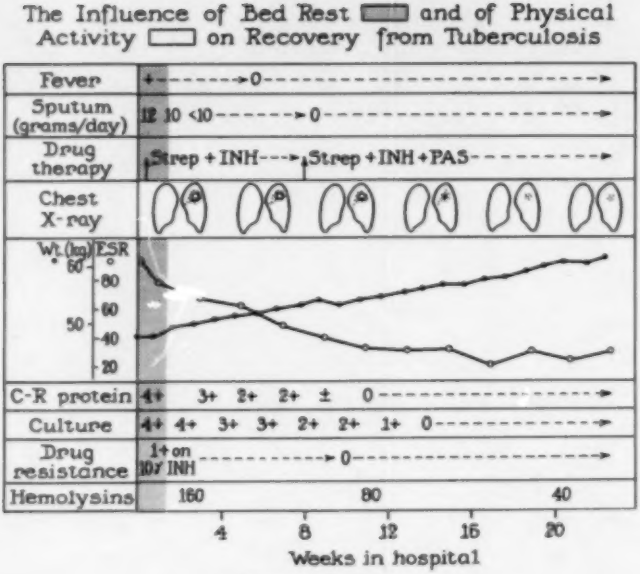
Her recovery was uncomplicated except for an increase in weight which was distressing to her. Therefore, in May, 1956, a reducing diet was instituted. She was discharged on August 17, 1956, five and a half months after admission, and has continued to be free of any abnormalities on subsequent regular follow-up examinations. Streptomycin will be given for a period of one year and isoniazid will be continued indefinitely.



March 7, 1956

Case 20

August 13, 1956

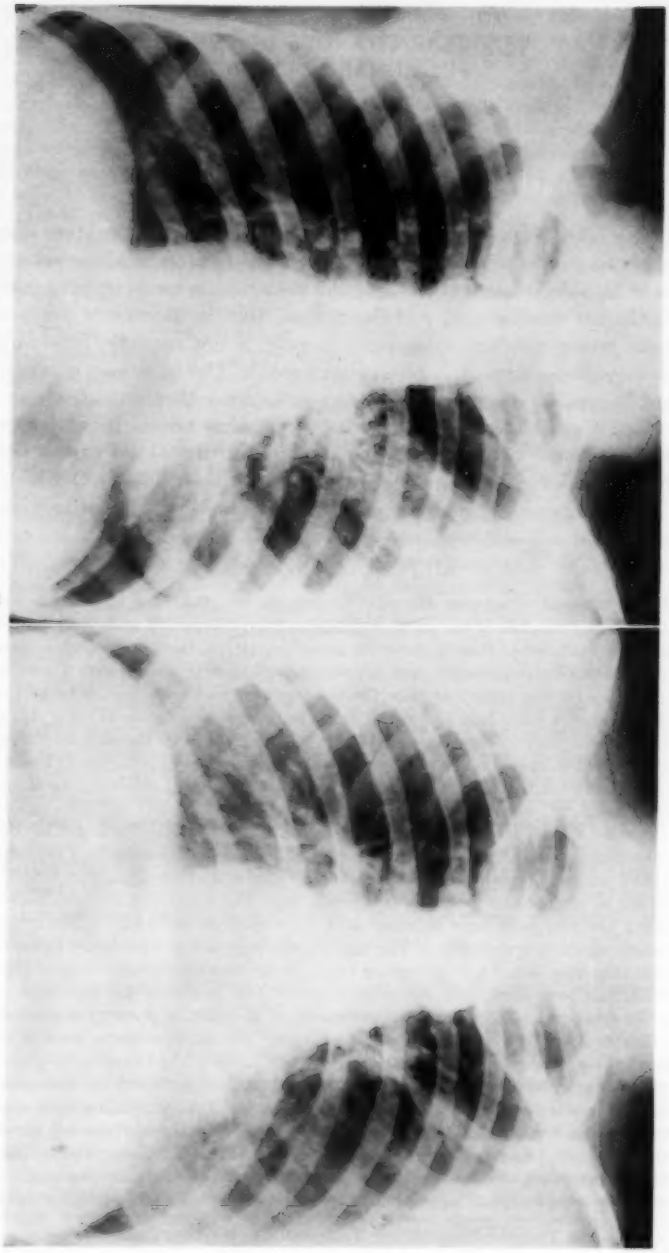


Case 21 (J. Mar.)

This patient, a 24-year-old married Negro woman, was admitted to the hospital on April 6, 1956, with a history of malaise, cough, hoarseness, and a 12 pound weight loss during the four months prior to admission. During 1953–1954, when she worked as a clerk in a hospital, she occasionally came in contact with patients on the tuberculosis wards. She had had yearly chest roentgenograms since 1953, the last of which in 1955 was said to be normal.

On examination she appeared thin and chronically ill. There was a slight inspiratory lag, and fremitus was decreased over the left lung fields. No persistent rales were heard.

Initial treatment consisted of streptomycin and isoniazid. Because organisms resistant to isoniazid were repeatedly cultured from her sputum, including a specimen obtained prior to therapy, 15 gm. daily of sodium PAS were added to the therapeutic regimen. The patient improved and was discharged on September 28, 1956, after approximately six months' hospitalization. Regular examinations in the outpatient clinic show no significant abnormalities.



April 6, 1956 Case 21 September 24, 1956

VASCULAR CHANGES IN THE LUNGS IN PULMONARY TUBERCULOSIS[1]

GODFREY L. GALE

(Received for publication August 27, 1956)

Introduction

It has been known for many years that chronic inflammatory disease in the lungs is associated with secondary endarteritis obliterans in the arterioles. In the case of established bronchiectasis, the endarteritis tends to progress in keeping with the irreversible nature of the disease. But, in the case of pulmonary tuberculosis, recent exudative disease is largely, if not entirely, reversible provided adequate chemotherapy is given soon enough. The questions immediately arise: What happens to the blood vessels in such cases? How quickly does endarteritis develop? Can it be prevented or its progression arrested if the tuberculosis is treated in time? Moreover, what changes do the blood vessels show in lung parenchyma which has once been the seat of tuberculosis, but which, now with the aid of chemotherapy, has undergone resolution to relatively normal lung tissue?

Plan of Investigation

In an attempt to answer these questions, 26 resected lung specimens were carefully studied. The branches of the pulmonary artery were injected with radiopaque fluids, and roentgenograms were taken to show the vascular pattern. Histologic studies were then made to demonstrate the presence and degree of endarteritis obliterans. These findings were correlated with the length of time the tuberculosis had been known to be present before chemotherapy was started and with the amount of antituberculous drug therapy that had been received prior to resection. Serial chest roentgenograms showed the extent of clearing of the process that had taken place under the influence of chemotherapy. These factors were all studied to try to find out as much as possible about the development of endarteritis in these cases.

Injection technique: Various techniques were tried to find the best one for demonstrating roentgenographically the vascular pattern in the resected specimens. The technique finally adopted was to take the resected specimen right after operation and inflate the bronchi with air to restore the specimen to normal size. The bronchi were then tied off. The pulmonary arteries were next injected with lipiodol at room temperature and tied off, and roentgenograms were obtained. The lipiodol was injected at a moderate pressure in order to distend the vessels but not to rupture them. If the roentgenograms showed that insufficient lipiodol had been used, more could be injected. The specimen was then sent to the laboratory for fixation and section in the usual way. The vascular pattern was not demonstrated satisfactorily in the roentgenograms in all cases, but the specimens were all studied histologically.

Specimens examined: Of the 26 specimens examined, 4 were from pneumonectomies, 13 from lobectomies, and 9 from segmental resections, the latter ranging from a single segment (the posterior segment of the left upper lobe) to three segments (the left basal segments in two instances). The resections were done between April, 1954, and June, 1955. During this period many other resected specimens were rejected as unsuitable for injection, either because they were too small or too badly damaged on being freed from the chest wall.

[1] From the Toronto Hospital for Tuberculosis, Weston, Ontario.

Duration of disease: The known duration of *clinical* disease prior to resection varied considerably. It was considered in each individual case in studying the histology. The shortest known duration was eleven months and the longest was ten years. The average duration was three years, with duration in half the cases of less than eighteen months. It was clearly impossible to fix the onset of clinical disease with absolute accuracy in all cases but, when all factors were taken into consideration, a fair estimate could often be made; as, for example, when an active, healthy young housewife suddenly developed symptoms and within a month had a diagnostic chest roentgenogram. She may have had a primary focus for some time but the onset of bronchogenic dissemination could be dated with some accuracy.

Institution of chemotherapy: An important factor in the development of endarteritis must be the interval between the onset of clinical disease and the beginning of antituberculous chemotherapy. In this series, 18 of the cases were clear-cut first-treatment cases, and the average interval between the onset of clinical disease and the commencement of chemotherapy was two and one half months. Four patients were receiving treatment within one month after the known onset of disease.

Duration of chemotherapy: In the Toronto Hospital for Tuberculosis, most patients with active pulmonary tuberculosis are started on streptomycin, 1 gm. twice a week, with sodium PAS, 14 gm., and isoniazid, 300 mg. daily, as soon as a sufficient number of sputum cultures have been planted. In the present series, all patients received this "triple-drug" regimen except two patients who for various reasons received only streptomycin and PAS. The duration of chemotherapy prior to resection varied from eight months to four years. The average duration of chemotherapy before resection was sixteen months and in only 4 cases had this exceeded eighteen months. In each case the patient had received what was believed to have been an adequate preoperative course of drugs, with sufficient time for exudative disease to resolve, although there has more recently been a tendency to advocate resection earlier if cavitation persists.

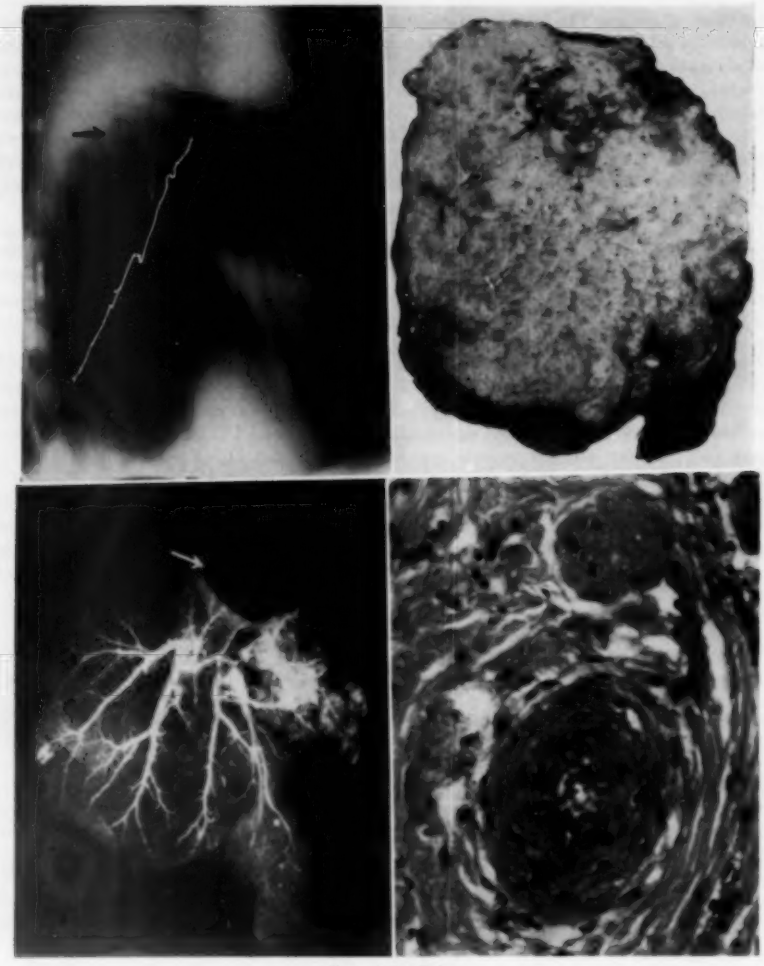
Observations

Case Reports

Case 1. D.L. was a 23-year-old married white woman. The known duration of pulmonary tuberculosis prior to resection was only sixteen months. She had been in excellent health up to that time. Upon admission to the sanatorium moderately heavy, scattered, cavitary disease was noted throughout the left lung. Chemotherapy was started two months after her first symptom. At the time of operation she had received chemotherapy for fourteen months and there had been good roentgenographic clearing of disease apart from a residual 3 cm. cavity in the left upper lobe. At thoracotomy scattered small nodules of disease were palpable throughout the left lung. A left upper lobectomy was performed, including the lingula. Injection of the resected specimen showed loss of the finer arterioles in the vicinity of the cavity, and histologic examination showed an extreme degree of endarteritis obliterans (figures 1 to 4.)

Comment: This example seems to show that a severe degree of endarteritis obliterans can develop in a relatively short time.

Case 2. A.B. was a 36-year-old white man. The known duration of pulmonary tuberculosis prior to resection was twelve months. The patient had had a normal chest film two years before admission. Upon admission to the sanatorium a 5 cm. tuberculoma with a few satellite nodules in the posterior segment of the left upper lobe was found. The rest of the lung appeared clear roentgenographically. The lesion changed very little after ten months of chemotherapy. At thoracotomy the disease was apparently limited to this one segment which was excised. Histologic examination did not show endarteritis at any point (figures 5 and 6).



Case 1 (D.L.)

FIG. 1 (Upper left). Left lateral planigram showing residual cavity in the posterior segment of the left upper lobe.

FIG. 2 (Upper right). Left upper lobe showing cavity.

FIG. 3 (Lower left). Roentgenogram of injected specimen showing loss of finer arterioles in the region of the cavity.

FIG. 4. (Lower right). Histologic section (× 320) showing marked endarteritis of one arteriole and probable obliteration of another.

Comment: In this case the localized tuberculoma with its satellite nodules was not associated with any endarteritis obliterans.

Case 3. P.O'L. was a 37-year-old white man. The known duration of pulmonary tuberculosis was thirteen months prior to resection. When the diagnosis was first made there was exten-

Case 2 (A.B.)

FIG. 5 (Top). The posterior segment of the left upper lobe showing a circumscribed tuberculoma.

FIG. 6 (Bottom). Histologic section (× 30) showing a normal arteriole in lung tissue adjacent to the tuberculoma.

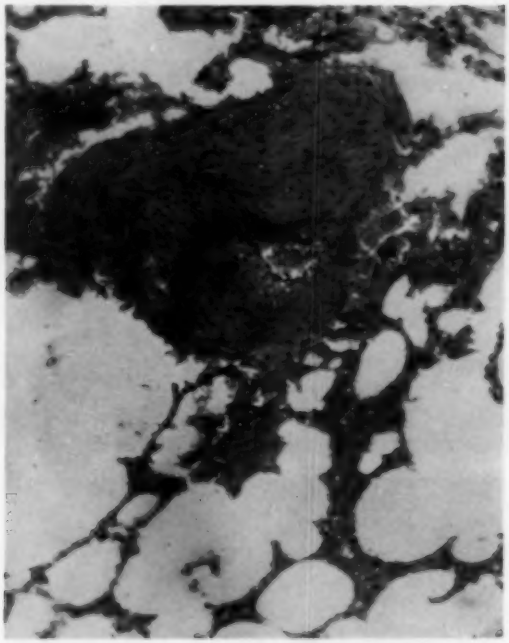


FIG. 7. *Case 3* (P.O'L.). Histologic section (× 80) showing marked endarteritis of an arteriole in normal lung tissue.

sive infiltration throughout the right upper lobe with cavitation. Chemotherapy was begun within a month of the patient's first symptom and drug treatment was given for a little more than twelve months before operation. Moderate clearing took place but he was left with residual cavitation. A right upper lobectomy was carried out. The specimen showed that a severe degree of endarteritis had persisted in lung parenchyma which had otherwise returned to normal (figure 7).

Comment: In spite of early institution of chemotherapy and roentgenographic clearing of disease, the endarteritis persisted. It is probably irreversible once it has occurred.

Case 4. A.H. was a 42-year-old white man. The diagnosis of pulmonary tuberculosis was first made eight years prior to resection. Extensive disease involved most of the right lung, with a thickened pleura as the result of an old pneumothorax. A right pneumonectomy was performed after eight months of chemotherapy. The histology was remarkable for the fact that the endarteritis was chiefly limited to the finer arterioles. The medium arterioles often escaped entirely (figure 8).

Comment: The endarteritis obliterans is usually most extensive when associated with bronchiectasis or in a collapsed lobe under a thoracoplasty. This case,

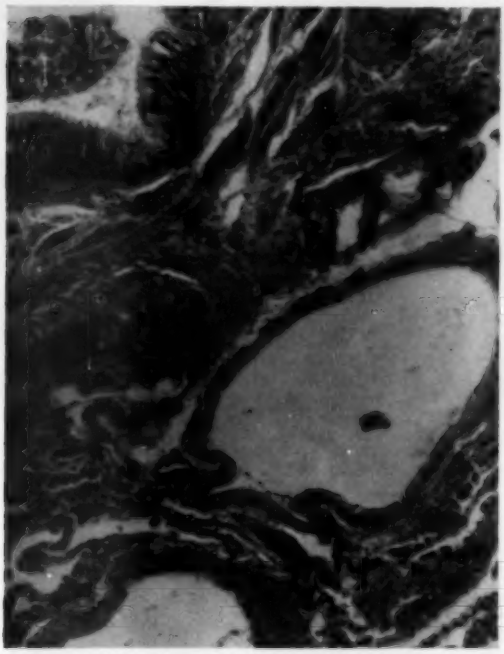


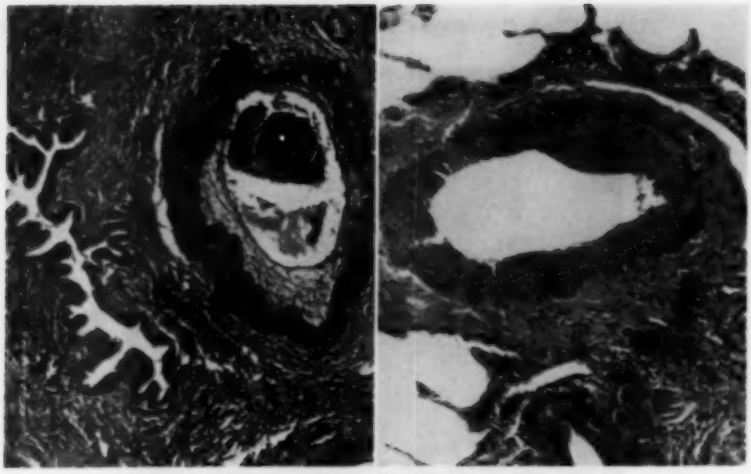
FIG. 8. *Case 4* (A.H.). Histologic section (× 80) showing normal arterioles in long-standing pulmonary tuberculosis.

however, shows that the degree is unpredictable and may be limited, even in old chronic disease.

Case 5. C.E. was a 42-year-old married white woman. The known duration of pulmonary tuberculosis was ten years prior to resection. Bilateral disease was present. Following nineteen months of chemotherapy there was a good response in the left lung but a poor response in the right, and consequently a right upper lobectomy was done. In figure 9 marked endarteritis of an arteriole is shown near a small bronchus surrounded by diseased lung; and in figure 10 endarteritis of an arteriole adjacent to a bronchus on one side may be seen, with normal lung parenchyma on the other side.

Comment: It was noted in this and in several other instances that the endarteritis was most marked in arterioles closely adjacent to bronchi. From their relative size they were thought to be pulmonary rather than bronchial arteries.

Case 6. L.D. was a 19-year-old single white woman. The known duration of pulmonary tuberculosis prior to resection was nineteen months. Upon admission to the sanatorium there was dense disease in the upper part of the right lung, with a large cavity medially. Chemotherapy was begun four months after the patient's first symptom and there was good clearing roentgenographically. After fifteen months of chemotherapy cavitation was still present, however, and a right upper lobectomy was performed. A pair of blood vessels at



Case 5 (C.E.)

FIG. 9 (Left). Histologic section (× 80) showing marked endarteritis in an arteriole adjacent to a bronchus surrounded by grossly diseased lung tissue.

FIG. 10 (Right). Histologic section (× 80) showing moderate endarteritis in an arteriole adjacent to a bronchus on one side and normal lung tissue on the other.

FIG. 11. *Case 6* (L.D.). Histologic section (× 30) showing endarteritis of an arteriole with a normal vein, adjacent to a caseous focus.

the edge of a tuberculous focus is shown in figure 11. One vessel, the arteriole, shows marked endarteritis. The other vessel, the vein, does not show any disease.

Comment: The endarteritis involves the arterial system, especially medium-sized arterioles, but not the veins.

Discussion

This report is not regarded as a presentation of any new facts. It seeks rather to re-assess known facts about endarteritis of the pulmonary vessels in pulmonary tuberculosis and to offer a concept of the importance of these facts in relation to the chemotherapy of the disease and to pulmonary resection.

The changes that occur in the walls of the arterioles in the lungs as the result of long-standing inflammation have been known for a hundred years. Arterioles of any size may be involved from the largest to the smallest. There is a fibroblastic proliferation of the intima which may be regular or eccentric. Edema of the vessel wall and infiltration with lymphocytes and neutrophils ultimately lead to scar production. These changes should be distinguished from those due to pulmonary hypertension and to the "collagen" diseases. Neither of these conditions was present, so far as is known, in this series of cases.

Endarteritis is often most advanced in old chronic cases of tuberculosis, especially under a thoracoplasty, as shown by Auerbach (1), or when it is associated with bronchiectasis (2). The changes are permanent even if subsequent roentgenographic clearing occurs (3). It would seem reasonable to suppose that this endarteritis results in permanent loss of pulmonary function, and this has in fact been demonstrated by many workers. Further, as Maier pointed out in the discussion of a paper by Hurst, Dressler, and Denst (4), the loss of pulmonary function depends on the extent of the original pathologic process. An old, bilateral, diffuse lesion which has apparently cleared well roentgenographically may show a greater reduction in pulmonary function than a case in which there are perhaps two destroyed lobes. One should recall in this connection that there are not likely to be any useful anastomoses in the human subject between the pulmonary and the bronchial vessels, although communications certainly exist (5, 6).

One other fact requires emphasis. Endarteritis may confidently be assumed to be present in the majority of cases of pulmonary tuberculosis, but its extent is not entirely predictable. Occasionally it may be more extensive and severe than anticipated (7) or, for some unexplained reason, the vessels may escape relatively lightly (8). In this present series of 26 cases, 25 patients had moderate to severe endarteritis of the arterioles. In one case (*Case 4*), however, endarteritis of the finer arterioles was present, while the medium arterioles often escaped entirely. In another case (*Case 2*), in which a 5 cm. tuberculoma with a few satellite nodules was present, there was no endarteritis of the surrounding arterioles.

How rapidly does the endarteritis develop? The present investigation suggests that in certain cases it can develop early in the course of pulmonary tuberculosis and progress in a relatively short time to a marked degree. In 2 cases, for instance (*Cases 1* and *3*), chemotherapy was commenced two months and one

month, respectively, from the onset of the first symptoms. These patients may have had a quiescent primary focus previously, but the onset of bronchogenic spread and clinically recognizable disease was sufficiently abrupt to be able to date it with some accuracy. Nevertheless, in spite of the early institution of combined chemotherapy and good roentgenographic clearing of exudative disease, in both cases extensive and severe endarteritis was found in the resected specimens.

The importance of these observations is in connection with pulmonary resection. Oxygen uptake may be permanently reduced by the endarteritis even if ventilation remains unchanged. Such lung tissue may be left in the chest at resection to act as a "space-filler," but it must not be expected to contribute much to pulmonary function. This fact should also be kept in mind when considering pulmonary resections in borderline cases where it is feared that the operation might leave the patient a respiratory cripple. If a lung has at any time in the past been the seat of extensive tuberculosis, its usefulness should be regarded with grave suspicion even if it has apparently cleared well roentgenographically.

Summary

Twenty-six resected lung specimens were examined roentgenographically and histologically for endarteritis obliterans of the arterioles. An attempt was made to correlate the findings with the length of time the tuberculosis had been known to be present before chemotherapy was started and with the degree of roentgenographic clearing that had taken place at the time of resection.

It appears that a marked degree of endarteritis can develop in pulmonary tuberculosis in a relatively short period. This change is probably permanent even if roentgenographic clearing of exudative disease occurs under the influence of chemotherapy. It is more severe with widespread disease than with localized tuberculomata, but the degree of endarteritis is not entirely predictable.

Any portion of lung which has at any time been the seat of extensive tuberculosis has probably lost some degree of oxygen uptake permanently, no matter how well it ventilates or how well the lesion has apparently cleared roentgenographically. This should be taken into consideration in planning pulmonary resections.

Sumario

Alteraciones Vasculares en los Pulmones en la Tuberculosis Pulmonar

Veintiséis ejemplares pulmonares resecados fueron examinados roentgenográfica e histológicamente en busca de endarteritis obliterante de las arteriolas. Tratóse de correlacionar los hallazgos con el período de tiempo en que se sabía que había existido la tuberculosis antes de iniciarse la quimioterapia y con el grado de despejo radiográfico que había tenido lugar para la fecha de la resección.

Parece que puede presentarse una endarteritis acentuada en la tuberculosis pulmonar en un período de tiempo relativamente breve. Esta alteración es probablemente permanente aunque se observe despejo radiográfico de la enfermedad exudativa bajo el influjo de la quimioterapia. Si bien es más grave con la tuberculosis generalizada que con la localizada, no cabe predecir por completo la intensidad de la endarteritis.

Toda porción del pulmón que haya sido en alguna ocasión asiento de tuberculosis extensa ha perdido con toda probabilidad permanentemente alguna parte de la absorción de oxígeno no importa lo bien que se airee o lo bien que se haya despejado al parecer radiográficamente. Hay que tomar en cuenta esto al proyectar resecciones pulmonares.

Resume

Modifications vasculaires dans les poumons dans la tuberculose pulmonaire

Des examens radiologiques et histologiques ont été effectués sur 26 pièces de tissu pulmonaire réséqué, pour rechercher les lésions de l'endartérite oblitérante des artérioles. Il a été tenté d'établir une corrélation entre les signes observés et la durée de la période entre le moment où la tuberculose a été reconnue et l'institution de la chimiothérapie, ainsi que le degré de l'amélioration radiologique observée au moment où la résection a été effectuée.

Il a été constaté qu'un degré marqué d'endartérite pouvait se développer dans la tuberculose pulmonaire dans une période relativement brève. Cette altération est probablement irréversible, même lorsque la radiologie montre la disparition des exsudats sous l'influence de la chimiothérapie. L'endartérite est plus sévère quand l'affection est plus extensive que lorsque les tuberculomes sont localisés, mais le degré de l'endartérite n'est pas absolument prévisible.

Tout segment de poumon ayant été à un moment donné le siège de lésions tuberculeuses extensives a probablement perdu de façon permanente un certain degré de sa capacité de capter l'oxygène, même si la ventilation est satisfaisante et si les opacités radiologiques paraissent éliminées. Ce fait doit être considéré lorsqu'on établit le plan des résections pulmonaires.

Acknowledgments

The writer wishes to express his thanks to Dr. C. A. Wicks, Superintendent, Toronto Hospital for Tuberculosis, Weston, Ontario; and to Dr. F. G. Kergin and Dr. N. C. Delarue, Consultants in Surgery, for much helpful advice.

REFERENCES

(1) Auerbach, O.: Anatomic changes in the lungs following thoracoplasty, J. Thoracic Surg., 1941, *11*, 21.

(2) Gobbel, W. G., Gordon, J., and Digman, G. J.: The pulmonary artery in bronchiectasis, J. Thoracic Surg., 1951, *21*, 385.

(3) Barrett, R. J., Masaki, M., and Day, J. C.: Circulatory status of resected tuberculous pulmonary lobes, J. Thoracic Surg., 1954, *27*, 277.

(4) Hurst, A., Dressler, S. H., and Denst, J.: The relationship between pathological changes in blood vessels in resected lobes and lungs as correlated with pulmonary artery pressure changes recorded during cardiac catheterization, Dis. of Chest, 1953, *24*, 41.

(5) Cockett, F. B., and Vass, C. C. N.: The collateral circulation to the lungs, Brit. J. Surg., 1950, *38*, 97.

(6) Marchand, P., Gilroy, J. C., and Wilson, V. H.: Anatomical study of bronchial vascular system and its variation in disease, Thorax, 1950, *5*, 207.

(7) Denst, J., Hurst, A., and Dressler, S. H.: A histologic study of the blood vessels in surgically resected tuberculous lungs, Am. Rev. Tubere., 1951, *64*, 489.

(8) Cicero, R., and Celis, A.: Ante-mortem and post-mortem angiography of the pulmonary arterial tree in advanced tuberculosis, Am. Rev. Tubere., 1955, *71*, 810.

AIR HYGIENE IN TUBERCULOSIS: QUANTITATIVE STUDIES OF INFECTIVITY AND CONTROL IN A PILOT WARD[1]

A Cooperative Study Between the Veterans Administration, The Johns Hopkins University School of Hygiene and Public Health, and the Maryland Tuberculosis Association

RICHARD L. RILEY,[2] WILLIAM F. WELLS, CRETYL C. MILLS, WALENTY NYKA, AND ROSS L. McLEAN

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INTRODUCTION

From both clinical and laboratory studies has come evidence which converges on the concept that pulmonary tuberculosis may be primarily air-borne. In "open" cases, vast numbers of tubercle bacilli are harbored in the sputum; violent expiratory processes such as coughing and sneezing atomize myriads of tiny droplets into the air; nuclei remaining after instantaneous evaporation of these droplets stay air-borne until breathed or vented; infective droplet nuclei when breathed by animals are implanted on susceptible lung tissue where they induce tubercles. If the chain of events suggested by these findings is substantially correct, then the possibility of breaking the chain by sanitary control of contaminated indoor air deserves consideration. In the light of the outstanding success of sanitary control of water-borne and insect-borne parasites, air hygiene offers a major challenge to public health.

Dr. John B. Barnwell, Director of Research and Education for the Veterans Administration, is the man who initiated the study of air hygiene in tuberculosis and nurtured this scientific baby to its present adolescent stage. Dr. Barnwell's interest, in turn, was largely the result of Mr. William F. Wells' impressive laboratory studies at Harvard and the University of Pennsylvania (1). These studies clearly indicated the need for a full-scale test in a pilot ward. A suitable place for such a test was finally found at the Veterans Administration Hospital in Baltimore, and Mr. Wells and his assistant, Mrs. Cretyl C. Mills, moved their base of operations there. Through the extraordinary generosity of the University of Pennsylvania they brought with them Mr. Wells' calibrated animal exposure chamber and other pieces of apparatus which have been of inestimable value in the present studies. Dr. Riley became associated with the project because of his early interest in Mr. Wells' work in the 1930's and because of his connection with The Johns Hopkins University School of Hygiene and Public Health. The Maryland Tuberculosis Association joined in by contributing funds and encouragement at a crucial moment. Dr. Ross L. McLean, Director of Professional Services at the Veterans Hospital, coordinates the laboratory and clinical aspects of the project and provides essential liason between the line and the staff within the VA. Dr. Walenty Nyka, pathologist of the hospital, provides expert advice in his field and an impartial check on estimates of the number of tubercles in animal lungs. Dr. Bruce Armstrong gave invaluable support when the Baltimore project was in its infancy.

[1] Presented at the meeting of the Eastern Section of the American Trudeau Society in Schenectady, New York, November 9, 1956.

[2] Present address: The Johns Hopkins School of Hygiene and Public Health, 615 N. Wolfe St., Baltimore 5, Maryland.

The present report deals with some of the studies which have been performed in the pilot ward to date. These preliminary steps include: control of ventilation; control of hazards from escaping bacteria; control of infection and disinfection within the ward; comparison of the specific infectivity of different strains of tubercle bacilli; design, construction, and operation of a special animal exposure chamber; and finally, calibration of the pilot ward. Each step has been essential in preparing for the major objective, which is to determine whether animals can be infected by breathing the air from the pilot ward when it is occupied by human patients with open tuberculosis, and whether such air-borne infection, if achieved, can be eliminated by means of air hygiene.

Observations

Figure 1 is a schematic picture of the pilot ward and its ventilating system. Six single rooms in a row open onto an inner corridor which in turn opens onto the main corridor of one of the regular hospital wards. Standard ultraviolet light fixtures which irradiate the air above head level are installed in each room. The pilot ward is on the top floor of the hospital and its own private air-conditioning system is located in a penthouse immediately overhead. As now arranged, exhaust air on its way to being vented outdoors passes through a large animal exposure chamber. Other modifications which were necessary will be described below.

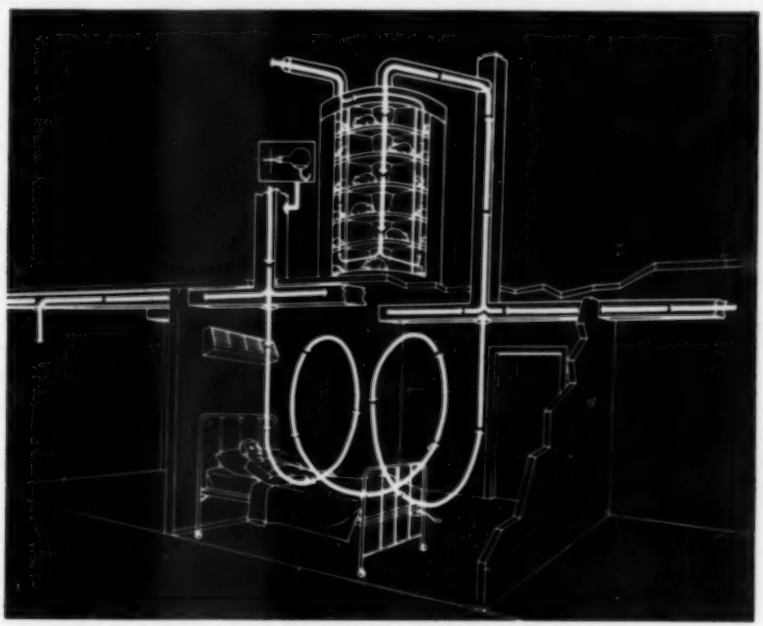

Fig. 1. Schematic diagram of pilot ward and animal exposure chamber.

Figure 2 is a photograph taken from the inner corridor. The ultraviolet light fixtures over each door may be seen. The main hospital corridor is to the left. In figure 3 is shown the interior of one of the rooms with the ultraviolet fixture for irradiating the upper air.

Control of Ventilation

Control of ventilation was essential because the infectivity of ward air depends upon the amount of dilution of infective particles with outside air. The first step in preparing the pilot ward for the experiments was to reduce the intake of fresh air both by closing off the intake grill and by changing from an open circuit to a recirculation system. Determination of the total amount of fresh air entering the system posed a difficult technical problem because air entered through innumerable small leaks, particularly at junctions in the ductwork and around doors and windows. Furthermore, the pressure in part of the system was slightly above atmospheric and in other parts slightly below atmospheric. Mr. A. B. Hubbard of the Air Conditioning Division of the General Electric Company made a preliminary investigation of this situation, and estimates of total fresh air intake were then made by Mr. Carl W. Coblenz of the National Bureau of Standards, using a helium-dilution method. Without the help of these engineers, quantitative studies of infection and disinfection in the pilot ward would not have been possible.

With the helium-dilution tests used to monitor progress, control of ventilation

FIG. 2. Photograph of inner corridor of pilot ward.

FIG. 3. Photograph of one of the rooms in the pilot ward.

was finally accomplished by a combination of direct and indirect approaches. Plastic curtains were stretched over the windows and other leaks were plugged as well as possible with Scotch® cellophane tape, cardboard, and plastic sheets. Then the fan in the ventilating system was slowed in order to lower the pressure within the system and thus reduce the amount of air passing through any remaining cracks. This changed the aerodynamics of the system, but the ventilation characteristics now satisfied the requirements of the program. Entering fresh air amounted to approximately 213 cu. ft. per minute, or roughly 40 cu. ft. per minute per patient. This value exceeds good public school ventilation standards, yet causes a dilution of contamination which is low enough, it is believed, to permit measurement of the infectivity of "open" cases of pulmonary tuberculosis. That this belief is more than a pious hope will become apparent after quantitative studies dealing with artificial contamination of the ward air are described.

Control of Hazards from Escaping Bacteria

Control of hazards from escaping bacteria depends partly upon minimizing the outward movement of air through leaks in the system and partly upon posi-

tive means of destroying organisms before they can enter the rest of the hospital. After numerous modifications, the ventilating system was so balanced that virtually all air left the system through the exhaust duct and very little through leaks in the system. This aerodynamic control reduced the likelihood of organisms escaping into the rest of the hospital, but did not provide a guarantee that occasional organisms might not escape from parts of the system where the pressure exceeded atmospheric.

The final guarantee was provided by a bactericidal barrier which enveloped the entire pilot ward and its air-conditioning system. Fortunately, the section of duct where the pressure was highest, hence the possibility of outward leakage greatest, was in the penthouse. The air surrounding this ductwork could be irradiated directly with ultraviolet light. Naked bactericidal burners attached to the high ceiling flooded the whole space with germicidal radiation which, it is believed, no parasite could survive for an instant. The inner corridor separating the ward from the hospital was converted into a bactericidal air lock by light curtains hung over the door to the rooms and to the outer corridor. The space between the ward ceiling and the penthouse floor, which carries the distributing ducts, was also heavily irradiated. No air escaping from these ducts could reach the hospital without passing through a deadly barrage of germicidal radiation. Indeed, the ceiling blocks at several points in the main corridor around the ward were replaced with grids through which ultraviolet light could stream downward, tending to convert these corridors from channels conveying air-borne germs into air purification chambers.

The bactericidal tightness of this all-embracing barrier was checked repeatedly by atomizing high concentrations of *E. coli* into the air at the conditioner and sampling wherever it seemed remotely possible that escaping organisms might be picked up. The absence of *E. coli* from samples of air elsewhere in the hospital, when thousands per sample were collected from pilot ward air, satisfied responsible authorities as to the tightness of control of the hazard of escaping bacteria.

Control of Infection and Disinfection Within the Ward

In the first tests, a broth culture of *E. coli* was atomized into the air-conditioning system and, after allowing time for equilibrium conditions to be reached, organisms in the exhaust air were sampled with the Wells air centrifuge. Tests were made with and without the ultraviolet lights on in the rooms. Only 2.5 per cent as many test organisms were recovered when the ultraviolet lights were on as when they were off. These findings immediately demonstrated the over-all efficiency of ultraviolet disinfection within the ward. However, additional tests in which samples were taken within individual rooms gave inconsistent results which were best explained as due to inadequate mixing of the air in the upper and lower parts of the rooms. The importance of circulation of air within each room, carrying infective particles quickly from the lower unirradiated to the upper irradiated zone, was brought strongly to attention by these tests. During subsequent animal experiments fans were placed in each room to circulate the air. At present this internal circulation is accomplished by a modification of the air-conditioning system.

Bovine tubercle bacilli were first atomized into the air-conditioning system of the pilot ward in March, 1955. Twelve rabbits were placed in one of the rooms, all the ultraviolet lights were turned on, the atomizer flask was filled with culture filtrate, and the atomizer was turned on. The rabbits breathed the air with the ultraviolet lights on for two hours. Then the atomizer was turned off and 6 animals were removed. All the ultraviolet lights in the rooms were then turned off, the atomizer was restarted, and the 6 remaining rabbits breathed the infected air for three hours. Again the atomizer was turned off and all the lights were turned on for purposes of decontamination.

Eighteen days after exposure, one of the second group of animals died, apparently from an intercurrent infection. A few minute specks on the lungs looked suspiciously like tubercles. The first one examined contained acid-fast organisms, and it was thus known that the animals had been successfully infected. The remaining rabbits from this group were sacrificed on various occasions, and the numbers of macroscopic tubercles in the lungs may be seen in table 1. An average of seven tubercles was present in each of the 5 rabbits in which accurate counts could be made. None of the rabbits breathing irradiated air showed any sign of infection. Five were sacrificed and showed no gross evidence of tubercles. The lungs of 3 animals were submitted to the Armed Forces Institute of Pathology for examination, and no tubercles or acid-fast organisms were found. A guinea pig inoculated with the ground tissue from another rabbit has failed to develop tuberculous infection. The final rabbit is still alive and well more than a year and a half after exposure. Thus, disinfection as well as infection has been demonstrated in the pilot ward.

Specific Infectivity of Different Strains of Tubercle Bacilli

The next phase consisted of standardizing the procedure in order to perform more accurate quantitative studies. The first step involved recovery of a strain of bovine tubercle bacilli of known infectivity. On the theory that the most highly infective organisms would be those which had actually produced tubercles in the rabbits, several cultures were recovered from resected tubercles. Five of these cultures were atomized into the calibrated inhalation apparatus and their infectivity for rabbits was compared. In each case organisms in the air which the rabbits breathed were collected in buffer solution in the air centrifuge, aliquots of the buffer solution were planted on solid media, and the colonies which subsequently developed were counted. Each colony represented a viable particle in the air which the rabbits had breathed. These counts were compared with the

TABLE 1

INFECTION AND DISINFECTION OF PILOT WARD

Ultraviolet Lights in Rooms	Tubercles per Rabbit*					
On	0	0	0	0	0	0
Off	[2+]	4	5	7	9	10

* All 12 rabbits exposed in Room 2.

[ ] Indicates interference with accurate count by intercurrent infection.

number of tubercles developing in the rabbits' lungs. In table 2 the average number of viable particles breathed, as determined by the cultural method, is compared with the average number of tubercles counted in the lungs. The first culture was called Ravenel VA because of the high ratio of tubercles to colonies. The apparent excess of tubercles over colonies is attributed to technical inaccuracies. Within the limits of error of the methods, each viable nucleus which was inhaled produced a tubercle in the lungs. Ravenel VA is now used as the standard strain and, when grown and prepared according to standard procedures, can be used for quantitative studies.

When patients occupy the pilot ward, they will produce human strains of tubercle bacilli and not Ravenel VA. Accordingly, it was important to compare the behavior of human strains with the standard strain. Because of the limited number of untreated "open" cases among patients entering the hospital, the writers were particularly interested in determining whether patients producing drug-resistant organisms would be suitable test subjects. This decision hinged primarily on the infectivity of the patient's organisms as compared with Ravenel VA.

To date one isoniazid-resistant strain has been tested, and the results have yielded two major surprises of considerable importance. Culture filtrate containing a known concentration of organisms produced, when atomized, many times more than the expected number of viable nuclei, raising the possibility that this strain was more resistant than Ravenel VA to the trauma involved in changing from the aqueous to the air-borne state. In comparison with Ravenel VA the human strain produced about four times as many tubercles in guinea pigs. This important experiment is being repeated.

The second surprise related to the progress of the disease in the infected animals. There was suggestive evidence, based on the appearance of the lungs of the one guinea pig that was permitted to live for three months, that this drug-resistant human strain might be relatively avirulent. This finding is mentioned only as an indication that the technique of air-borne infection has important applications in the study of virulence and immunity.

TABLE 2

SPECIFIC INFECTIVITY OF DIFFERENT STRAINS OF TUBERCLE BACILLI

Strain	Animal	Average Number of Tubercles in Lungs	Average Number of Infective Particles Breathed*
Ravenel VA	Rabbit	50.0	30
2	Rabbit	0.0	0
3	Rabbit	9.0	450
4	Rabbit	120.0	250
5	Rabbit	0.5	50
Human†	Rabbit	1.0	5,000
Human†	Guinea pig	200.0	5,000

* Estimated by culture from air samples.

† Isoniazid-resistant strain.

From the point of view of the air hygiene study, the important finding was that guinea pigs exposed to drug-resistant organisms developed macroscopic tubercles in their lungs. This suggests that it may be possible to use patients producing drug-resistant organisms as sources of air-borne infection in the next phase of the study.

Design, Construction, and Operation of a Special Animal Exposure Chamber

In order to estimate the mean discharge of infective air-borne particles per "open" case of human pulmonary tuberculosis, it is necessary to sample infective droplet nuclei from large quantities of air over a long period of time. For this task the lungs of susceptible animals are far superior to any direct cultural technique. Because of the high dilution of the infective particles in the air and the small respiratory minute volume of a guinea pig, it is expected that it will be necessary to expose 150 to 200 guinea pigs for one year in order to obtain a statistically significant number of infections.

To accomplish this aim, a suitable animal chamber had to be devised (figure 4). A special cage arrangement with a number of unique features was devised by Mr. Wells. Without elaborating on its aerodynamics, its sewage disposal system,

FIG. 4. Photograph of animal exposure chamber with cage baskets removed. Note the small calibrated exposure chamber in the background.

and its continuous water supply, it can be said, on the basis of four months' experience, that animals have remained healthier in the exposure chamber than in ordinary cages. It is assumed that they will not all remain free of tuberculosis when the ward is occupied by infectious patients, for all the special features of the exposure chamber are incidental to the primary purpose, which is to facilitate the uniform exposure of guinea pigs to air from the experimental pilot ward.

Calibration of the Pilot Ward

The final step in the demonstration of the suitability of the pilot ward for measurement of infectivity of "open" cases was calibration of the exposure chamber. In this the investigators were helped by Mr. Wells' past experience with a culture having approximately the same specific infectivity as Ravenel VA. (This strain is mentioned as Experiment VII described on page 117 of Mr. Wells' book *Airborne Contagion and Air Hygiene* (1).) A broth culture of Ravenel VA was prepared according to standard technique and undiluted culture filtrate was atomized into the air-conditioning system of the pilot ward. The concentration of organisms in the flask fluid was approximately ten times as high as in the diluted culture filtrate used in Experiment VII cited above. However, the fresh air entering the air-conditioning system was about 100 times greater than that which flowed through the laboratory inhalation chamber used in Experiment VII. The resulting concentration of organisms in the air was thus, by calculation, about one-tenth as great. On the other hand, the rabbits were exposed for thirty minutes in Experiment VII and for two hours in the pilot ward experiment, so that altogether four-tenths as many infective nuclei should have been breathed. In Experiment VII an average of 165 tubercles developed per rabbit, and four-tenths of 165 is 66. Therefore, 50 to 100 tubercles per rabbit were to be expected. As can be seen in table 3 the two rabbits exposed in the pilot ward acquired 61 and 104 tubercles, respectively, and two more animals in the exposure chamber developed 71 and 72 tubercles. These results demon-

TABLE 3

CALIBRATION OF PILOT WARD

Ultraviolet Lights in Rooms	Room						Exposure Chamber*
	1	2	3	4	5	6	
	Tubercles per Rabbit						
Off		61			104		71, 72
	Tubercles per Guinea Pig						
Off	28	34, 45	23	20	20, 54	?	24, 12 30, 31 26, 22 7, 29

* Exhaust air containing a mixture from all rooms.

? Death before presence or absence of tubercles evident.

strate remarkable control of all of the factors involved in quantitative air-borne infection in the pilot ward.

Another important feature of this experiment was the close approximation between the number of tubercles acquired by animals in the ward and in the exposure chamber. Rabbits in the ward averaged 82 tubercles as opposed to 71 in the exposure chamber, and averages for the guinea pigs were 32 in the ward against 22 in the exposure chamber. This indicated that the air in the exposure chamber was for practical purposes equivalent in infectivity to the air in the ward itself. Furthermore, the rabbits developed on the average about three times as many tubercles as the guinea pigs, as would be expected from the fact that rabbits breathe about three times as much air per minute.

The crucial nature of the information provided by this experiment can be seen from the following calculations. Culture filtrate was atomized at a rate of about three drops per minute, and tubercles were produced in guinea pigs at a rate of approximately 0.2 tubercles per minute. Thus three drops of atomized culture filtrate produced an average of 0.2 tubercles per guinea pig. If 175 guinea pigs had been in the exposure chamber, three drops of atomized culture filtrate would have produced approximately 175 times 0.2 or 35 tubercles. In the next stage of the study when human patients will serve as producers of infective nuclei there will be about 175 guinea pigs in the exposure chamber, and it is hoped to have patients whose sputum contains tubercle bacilli in approximately the same concentration as the culture filtrate which was atomized in this experiment. If the patients' organisms have approximately the same specific infectivity as Ravenel VA, it can be anticipated that something of the order of 35 guinea pigs will be infected by the time the patients have atomized three drops of sputum. The study is planned to run for one year to give ample opportunity for the necessary conditions to be fulfilled. If in one year the patients produce as many infective nuclei as the atomizer produced in one minute, the study will provide a quantitative demonstration of the infectivity of humans with tuberculosis.

In conclusion, attention should be turned from infection to disinfection. From the point of view of air hygiene, the exciting part of the story is the demonstration that experimental animals can be protected against air-borne tuberculosis in a hospital ward. Those of us with a speculative turn of mind wonder whether air hygiene may advance as far in the next half century as water sanitation did in the last half century.

Summary

A six-room pilot ward has been adapted to the quantitative study of the infectivity of human patients with "open" pulmonary tuberculosis. The amount of fresh air entering the closed circuit air-conditioning system of the ward was carefully regulated and estimated to show the volume of air into which air-borne particles would be diluted. Each room was equipped with fixtures for irradiating the upper air with ultraviolet light. After artificial contamination of the air with *E. coli*, the effectiveness of the ultraviolet lights in killing the air-borne

organisms was demonstrated. Similar studies were then performed after artificial contamination of the air with bovine tubercle bacilli. A germ-tight ultraviolet envelope surrounded the ward to prevent escape of live organisms into the rest of the hospital. In these experiments animals served as samplers of air-borne infection, the number of tubercles developing in the animals' lungs indicating the approximate number of infective particles inhaled. The effectiveness of ultraviolet lights in killing air-borne tubercle bacilli was demonstrated. A separate experiment showed that a human strain of tubercle bacillus was probably even more infective than the bovine strain when atomized into the air. An exposure chamber to house 180 guinea pigs was then constructed and placed so that exhaust air from the ward passed through it before being vented. The air in this chamber was demonstrated to be nearly equivalent in infectivity to ward air. The pilot ward is now adequately calibrated so that the infectivity of human patients with pulmonary tuberculosis can be estimated from the number of guinea pigs which become infected from breathing air that is exhausted from the ward.

Sumario

La Higiene Aérea en la Tuberculosis: Estudios Cuantitativos de la Infectividad y del Dominio en una Sala "Pilota"

Una sala experimental de seis cuartos fué adaptada para el estudio cuantitativo de la infecciosidad de sujetos humanos que padecían de tuberculosis pulmonar "abierta." Graduóse cuidadosamente la cantidad de aire puro que penetraba en el sistema de circuito cerrado de acondicionamiento de la sala, calculándose de modo que mostrara el volumen de aire en que se diluirían las partículas transportadas por el aire. Cada aposento fué provisto de instalaciones destinadas a irradiar la porción superior del aire con luz ultravioleta. Después de la contaminación artificial del aire con *E. coli*, se comprobó la eficacia de las lámparas de luz ultravioleta para destruir los microbios transmitidos por el aire. Luego se ejecutaron estudios semejantes después de la contaminación artificial del aire con bacilos tuberculosos bovinos. Se circundó la sala con una envoltura ultravioleta a prueba de gérmenes para impedir el escape de microbios vivos al resto del hospital. En estos experimentos, usáronse animales como testigos de la infección transmitida por el aire, indicando el número de tubérculos que se formaban en los pulmones de aquéllos el número aproximado de partículas infecciosas inhaladas. Quedó comprobada la eficacia de la lámparas de luz ultravioleta para destruir los bacilos tuberculosos transmitidos por el aire. Otro experimento demostró que una cepa humana del bacilo tuberculoso era probablemente aun más infecciosa que la bovina al ser pulverizada en el aire. Luego se construyó una cámara de exposición capaz de acomodar 180 cobayos, colocándose de manera que la atravesara el aire usado antes de ventilarse. Se demostró que el aire de esta cámara casi equivalía en infectividad al de la sala. La sala experimental está ya calibrada adecuadamente de modo que cabe calcular la infectividad de los sujetos humanos que padecen de tuberculosis pulmonar por el número de cobayos que se infectan al respirar el aire expulsado de la sala.

Resume

Hygiène de l'air dans la tuberculose: Etude quantitative de la contagiosité et son contrôle dans une salle "pilote"

Une salle d'hôpital expérimentale comportant six chambres a été adaptée à l'étude de la contagiosité de cas humains de tuberculose pulmonaire "ouverte." La quantité d'air frais pénétrant dans le circuit fermé d'air climatisé de la salle était minutieusement réglée

et déterminée, afin d'évaluer le volume d'air dans lequel les particules transportées par l'air seraient dispersées. Chaque chambre comportait un système d'irradiation de la couche d'air supérieure par l'ultra-violet. Après contamination artificielle de l'air par *E. coli*, l'efficacité germicide des ultra-violets fut démontrée pour les particules dispersées dans l'air. Des études analogues furent alors entreprises après contamination de l'air par des bacilles tuberculeux bovins. Un barrage de rayons ultra-violets, impénétrable pour les germes vivants, prévenait leur passage de la salle expérimentale au reste de l'hôpital. Dans cette expérimentation, des animaux servaient à évaluer la contamination par l'air; le nombre de tubercules se développant dans les poumons de l'animal indiquant approximativement le nombre de particules infectieuses inhalées. L'efficacité germicide sur les bacilles tuberculeux dispersés dans l'air fut démontrée. Une expérimentation distincte a montré qu'une souche de bacille tuberculeux humain nébulisée dans l'air paraissait avoir une contagiosité plus élevée que la souche bovine. Une chambre d'exposition à la contamination pouvant recevoir 180 cobayes fut alors aménagée de façon à permettre à l'air s'échappant de la salle de traverser cette chambre avant ventilation. Il a été démontré que l'air de cette chambre avait un degré de contagiosité sensiblement égal à celui de la salle expérimentale. La salle "pilote" est désormais étalonnée de façon adéquate pour l'évaluation de la contagiosité des malades atteints de tuberculose pulmonaire, selon le nombre de cobayes infectés par inhalation de l'air s'échappant de la salle expérimentale.

REFERENCE

(1) Wells, W. F.: Airborne Contagion and Air Hygiene, published for The Commonwealth Fund by Harvard University Press, Cambridge, Massachusetts, 1955.

A SMALL EPIDEMIC OF TUBERCULOSIS[1]

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Introduction

The purpose of this paper is to present a history of a recent small epidemic of tuberculosis, to relate the way in which it was investigated and managed, and to attempt to assess the possible significance of this and similar outbreaks.

In July, 1955, the local health authorities and the Ontario Department of Health became alarmed by what appeared to be an outbreak of tuberculosis in a township in the northern part of the province. Nineteen active cases of tuberculosis had been reported among the 2,300 inhabitants of a French Canadian village and surrounding area. Twenty-one more cases were discovered during the course of the subsequent investigations, bringing to 40 the total in this village during the period of two years; most of these 40 cases occurred in adolescents and young adults.

The village in which the outbreak occurred is situated about 20 miles from a large and prosperous mining town. The standard of living is relatively high, the houses are mostly modern but, owing to the large size of the families, are somewhat overcrowded.

The social structure of the community proved surprisingly complex, at least four main groups keeping more or less to themselves. Furthermore, the community was split on the issue of alcohol, with a very active Alcoholics Anonymous group on the one side and an apparently fairly heavy consumption of alcohol on the other. These various barriers divided the older people, but the youngsters mixed more freely and frequently.

There were several places where infection could have been acquired. There were occasional trips in crowded cars to a dance hall or theater in neighboring villages; also the skating rink has a small shed used for rest and warmth.

The young people in the village, however, favored a small clean restaurant where a large number of them, including some with "open" tuberculosis, met regularly at least once or twice a week. Fifteen to twenty young people crowded into a confined space, singing, dancing, and drinking "pop," would seem to provide ideal conditions for the spread of infection.

This epidemic of tuberculosis is not the first encounter of this community with the tubercle bacillus. Infection and death from tuberculosis have occurred there for many decades. From the interviews with the older members of the community, it is apparent that tuberculosis was known there for so long as they can remember. The review of the death reports shows that there were six deaths from pulmonary tuberculosis in this township in the decade between 1936 and 1945.

[1] From the Division of Tuberculosis Prevention, Provincial Department of Health, Toronto, Ontario, Canada.

The present outbreak has to be viewed against the background of the over-all picture of the tuberculosis problem in the Province of Ontario. The provincial tuberculosis mortality rate for the year 1955 was 4.7 per 100,000. The rate of positive tuberculin reactors at the age of fifteen years is about 10 per cent in cities but only 4 to 5 per cent in rural areas. In mass community surveys one case of active tuberculosis is found among 2,500 roentgenographic examinations. These figures compare not unfavorably with those found in many parts of the United States and in most of the provinces of Canada.

Plan of Investigation

The main measures taken in order to investigate and control this outbreak were:

(*1*) Assessment of the whole position of tuberculosis in the community. This included the review of records of old cases of tuberculosis, as well as study of active cases arising during this outbreak. In addition, the patients were closely questioned about their activities, associates, and friends.

(*2*) A roentgenographic survey of the whole population more than five years old. This met with an unprecedented success, as 99.2 per cent attended. This was followed by:

(*3*) A tuberculin survey with subsequent periodic roentgenographic follow-up of the positive reactors and BCG vaccination of the negative reactors. Half of the population attended this survey.

(*4*) A complete census of the community.

Observations

Morbidity: During a two-year period, a total of 40 cases of active tuberculosis were discovered. Ten of these cases were diagnosed in the first year and 30 in the second year. Twenty-one of the 40 cases of tuberculosis arose in persons between the ages of fifteen and twenty-four years, with a rate of 5.5 per 100 (table 1). This is a heavy morbidity. One of every 19 persons in this age group was affected with clinically active tuberculosis.

The morbidity in the other age groups was considerably lower: 1 per cent in those less than fourteen years of age and 0.9 per cent in those more than twenty-five. This still represents a very high morbidity when compared with the data for other parts of the province. The tuberculin survey throws further light on the differing degree in which the various age groups were affected.

Tuberculin survey: Following the recommendations of the World Health Organization (1), only the single intradermal test with 5 tuberculin units was

TABLE 1

Morbidity in Different Age Groups

Age Groups	Number of Persons in Community	Number of Cases of Tuberculosis	Morbidity per 100 Population
0–14	1,087	11	1.0
15–24	381	21	5.5
25 and older	850	8	0.9
Totals	2,318	40	1.7

used. The result of the test was considered positive if after forty-eight to seventy-two hours there was induration of at least 5 mm. in diameter with surrounding erythema.

In 8 of the 1,135 persons tested, the reaction was considered doubtful. There were also 2 positive reactors who had previously received BCG vaccination. Excluding the above 10, there were 1,125 persons who gave unequivocal reactions to the test with 5 tuberculin units and no positive reactions due to previous vaccination.

The results are shown in table 2 and figure 1.

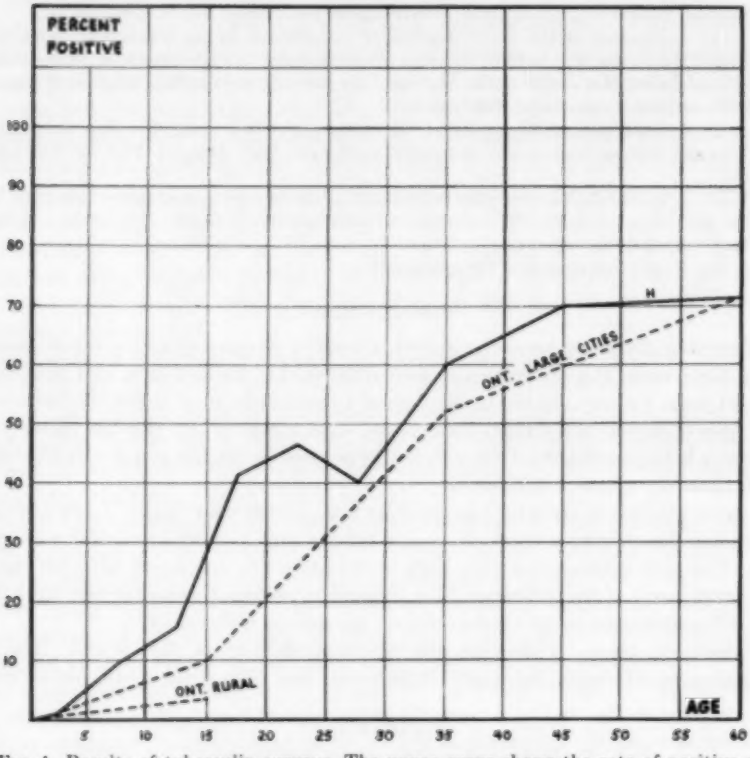

FIG. 1. Results of tuberculin surveys. The upper curve shows the rate of positive reactors in the village where epidemic took place; the lower one shows the rate in large cities in Ontario, Canada.

TABLE 2

TUBERCULIN SURVEY

Age	0–4	5–9	10–14	15–19	20–24	25–29	30–39	40–49	50 and older
Per cent positive	1	10	16	42	46	39	60	70	72

In figure 1, the upper curve shows the rate of positive reactors in the village; the lower one, inserted for comparison, shows the rate in large cities in Ontario (2). It would be better to compare this curve with that for rural areas. Unfortunately, however, the data here are limited to the first fifteen years of life. Inspecting the curve, it may be seen that the rate of positive reactors was somewhat higher than it was in the large cities but, on the whole, parallels the city rate except for the unexpected peak lying between fifteen and twenty-five years of age.

There was a very steep rise from 16 to 42 per cent in the rate of positive reactors seen when the ten to fourteen and the fifteen to nineteen age groups were compared. After the age of twenty the rate rose a little to 46 per cent in the next age group (twenty to twenty-four) and then dropped somewhat before resuming its upward course.

It is probable that these differences are mainly the result of recent "conversions." The opportunities for acquiring tuberculous infection during the recent outbreak, while evidently largest in the fifteen to twenty-four age group, were not limited to that group.

Thus, consideration of both tuberculous disease and tuberculous infection points to the same phenomenon. Both disease and infection, while affecting the whole community, were particularly heavy in the fifteen to twenty-four age group.

Types of disease: The various types of tuberculous disease discovered may be seen in table 3. As may be seen, the main part of the morbidity in this epidemic was due to primary tuberculous infection. This is naturally so in the first group with primary pulmonary tuberculosis. All of these 12 patients showed enlargement of the hilar lymph nodes on roentgenography. In 5, a primary focus was visible and, in one, there was evidence of collapse of a pulmonary segment. None of these lesions showed any calcification. Furthermore, 3 of the 12 patients were known recent tuberculin convertors and 5 had had normal chest roentgenograms eleven months previously. The next three disease types, pleurisy, meningitis, and miliary tuberculosis, are usually early complications of primary infection (3, 4).

In most of the cases of pulmonary tuberculosis there was no roentgenographic evidence of old primary infection. In many, there was a strong evidence, based on history, previous tuberculin tests, and previous roentgenographic findings, that the disease was of recent onset.

TABLE 3

TYPES OF DISEASE

Primary pulmonary tuberculosis	12
Pleurisy with effusion	3
Meningitis and miliary tuberculosis	3
Miliary tuberculosis alone	2
Skeletal tuberculosis	1
Chronic pulmonary tuberculosis (adult type)	19
Total	40

Thus it may be seen that this outbreak of tuberculosis was a result of widespread recent tuberculous infection, affecting particularly the adolescents and young adults.

Probable sources of infection: Pending further advances in bacteriology enabling the identification of "strains" of tubercle bacilli, it is impossible in an outbreak of this size to establish the sources and the chain of infection with strict scientific accuracy. Nevertheless, in a study of an epidemic, an effort should be made to find the probable course of events.

In the present investigation, a chest roentgenogram was obtained in more than 99 per cent of the population and all contacts of cases of tuberculosis were carefully examined and records relating to recent deaths were reviewed. It appears likely, therefore, that the sources of infection were among the 40 known cases of tuberculosis.

Two methods were used in the search. First, it was assumed that a potent source of infection is likely to have tubercle bacilli in the sputum on examination of a sputum smear. This assumption is based on the results of recent studies (5–7) which have suggested that patients with negative sputum smears, even when positive for tubercle bacilli on culture or gastric lavage, are not likely to spread infection. The proportion of positive tuberculin reactors among contacts of such patients does not differ appreciably from that seen in the general population. The second method was to inquire into the tuberculin status of the household associates of the patients with definite disease. It seems fair to assume that, if someone is infectious enough to be a real danger to the community, he can reasonably be expected to infect one or more members of his immediate family.

In this outbreak there were 5 subjects with sputum positive for tubercle bacilli on direct examination of a smear. In 3 of the subjects, tuberculin testing of their home contacts revealed some positive and some negative reactors. Two of the patients were hospitalized very early in the course of this outbreak and the third did not come into close contact with young people in the village.

The remaining 2 patients, a girl of nineteen and a boy of twenty, appeared most likely to be the main sources of infection in this epidemic. Their families (seven brothers and sisters of the girl and six brothers and sisters of the boy) were all positive reactors to tuberculin. Both of these patients—but particularly the boy—were very active socially and were frequent visitors to the restaurant favored by young people. It is of interest to note that the only other family in the village found to consist entirely of positive reactors was a young couple with four small children who owned the restaurant at the time of this outbreak.

Discussion

In the Canadian village mentioned above there was a small epidemic of tuberculosis involving chiefly the adolescents and young adults. Most of the 40 cases discovered were undoubtedly the result of recently acquired tuberculous infection.

A question which arose was whether the low native resistance could account for this outbreak. In the past, the introduction of tubercle bacilli for the first time into a primitive community has resulted in severe epidemics of tuberculosis.

The heavy mortality and morbidity seen in those epidemics had been ascribed to the low native resistance (8).

It is believed that low native resistance did not play a part in this epidemic. First: Tuberculosis was not a new disease in this district and deaths from tuberculosis had occurred there for many decades. The tuberculin reaction curve shows a high percentage of positive reactors in older age groups, almost certainly the result of infection acquired mainly much earlier in life. Second: The clinical picture of the individual cases of tuberculosis seen was certainly not suggestive of the disease as seen among primitive peoples. For example, in no single case was there a great enlargement of the lymph nodes. The roentgenographic picture of patients with primary complex or simple enlargement of hilar nodes did not differ in any way from that seen in communities exposed to tuberculosis for many generations. Hematogenous dissemination, while relatively common, was certainly an exception rather than a rule. Cases of pulmonary tuberculosis showed clinical and roentgenographic features and response to treatment similar to those seen in cases from other localities. Thus, it can be said that the low native resistance was not a significant factor in this epidemic.

Small epidemics of tuberculosis appear to be becoming more frequent in recent years, and perhaps not all of them are reported in the literature. Waring (9) reviewed five small epidemics occurring in various parts of the world. In Canada two such outbreaks were reported in the last four years, but there were at least two other outbreaks.

Maddison (10) reported an epidemic in New Brunswick in a village of about 800 and the surrounding parish of 750 people. The outbreak was brought to notice by the occurrence of 12 cases of erythema nodosum. Investigation of the whole community resulted in finding 45 cases of clinical tuberculosis, 30 of them in persons less than twenty-one years of age. The probable chain of infection was established, incriminating a patient with previously unknown, "open," far advanced pulmonary tuberculosis who spent most of the time sitting about a country store. He had probably infected a number of people at this common meeting place, including a fifteen-year-old boy who in a short time apparently became a source of infection in the village school.

In the epidemic described by Smith, McLellan, and Hiltz (11) the investigations commenced as a result of a case of tuberculous meningitis in a nine-year-old boy living in a very small rural community in Nova Scotia. This brought to light seventeen cases of tuberculosis, including another case of meningitis, one of pleural effusion, and four cases of pulmonary tuberculosis. During the subsequent period of five years, the number of new cases discovered in that small community rose to twenty-seven. The probable source of infection was a man with moderately advanced pulmonary tuberculosis living next to, and frequently visiting, the common store.

All of these small epidemics show certain common characteristics. In each there are, in addition to potent sources of infection, the places particularly suitable for the infection to spread, whether it be an unhygienic classroom (12), a restaurant, or a village store. Furthermore, the main part of the morbidity seen in these epidemics is due to recently acquired primary tuberculous infection;

they all resulted from the introduction of infection to tuberculin negative groups.

Thus, there appear to be three main factors necessary for the development of an epidemic of tuberculosis. These are:

A predominantly tuberculin negative population
The introduction of potent sources of infection
An environment suitable for the spread of infection

During the past few decades the tuberculin status of the population in most parts of North America has shown a steady change, with a decrease in the percentage of positive reactors. The speed of this process is really astonishing. For instance, a survey of school children living in a small urban and rural area in Ontario in 1923 (13) revealed 41 per cent of positive reactors between ten and fourteen years of age; the corresponding figures now vary between 3 and 8 per cent in different localities. Also in this province, Brink, Brown, and Gray (14) found that 47 per cent of nurses less than twenty years of age were tuberculin positive. In the intervening two decades that figure has decreased to about 15 per cent. Myers (15), from the neighboring state of Minnesota in the United States, reports the following percentages of the positive reactors among children between four and seventeen years of age attending parochial schools in Minneapolis: in 1926, 47.3 per cent; in 1936, 18.9 per cent; in 1944, 7.7 per cent; and in 1955, 3.9 per cent.

This rapidly changing picture of tuberculous infection can be attributed to the rising standard of living and particularly to better housing, on the one hand, and to the tremendous development of preventive measures with great achievements in the case-finding programs, on the other. It has become a justifiable source of pride to all those concerned with the prevention of tuberculosis.

Yet the story of the small epidemics shows that this rapid development of a tuberculin negative community may not be entirely beneficial; it is often forgotten that it means the development of a community without acquired resistance to tuberculous infection. Open undetected cases of tuberculosis are still being discovered by case-finding programs. Patients with old arrested infections who have been discharged from sanatoriums occasionally escape supervision and their disease reactivates after many years of apparent quiescence. In addition, opportunities for acquiring tuberculous infection are still very great in many areas of the world. With the considerable movements of the populations and development of travel in recent years, some members of the tuberculin negative communities may acquire their infection elsewhere and a few of them may in turn act as sources of infection on their return home.

The paramount importance of the environment in the problem of the spread of tuberculous infection has perhaps not received sufficient emphasis.

Tuberculosis under favorable environmental conditions is not usually a very infectious disease. Thus, in the community studied in this paper, in spite of a fairly widespread infection, about two-thirds of the population remained tuberculin negative. In a "well-to-do" suburb of London, Anderson, Grenville-Mathers, and Trenchard (16) found that only 6 per cent of infants less than one year of age who were home contacts of the infectious cases of tuberculosis were tuberculin

positive. This figure contrasts sharply with 90 per cent of positive reactors found by Price (17) in similarly exposed infants in Dublin.

Conditions of overcrowding, whether at home, in workshops, or in places of entertainment, appear essential for widespread infection with the tubercle bacilli. In the present instance, the habit of the young people of gathering regularly in the confined space of the local restaurant seems a vital factor in the development and the peculiar age distribution of this epidemic.

In conclusion, it appears reasonable to predict that minor epidemics of tuberculosis will arise from time to time among predominantly tuberculin negative communities. They will arise whenever undiscovered potent sources of infection arise in an environment suitable for the spread of infection. The experience of the last decades suggests that such epidemics will probably continue to be relatively rare.

Major epidemics appear unlikely. The high and rising standard of living, with good housing and hygienic workshops and places of entertainment, will largely control the environmental factor. The case-finding programs, if maintained and developed in spite of inevitably diminishing returns, will continue to keep down the number of actual or potential sources of infection.

There is no reason, however, why a major epidemic of tuberculosis should not occur if some disaster such as war or natural calamity should overtake us. If such circumstances should result in the crowding together of large numbers of people in confined spaces for prolonged periods and if a few open cases of tuberculosis were suddenly presented with a real opportunity to infect, the tuberculin negative community might well have to pay a heavy price for its lack of acquired resistance.

The lesson from these few small epidemics of tuberculosis appears to be that our general policy toward the prevention of tuberculosis may need revision. The need for protective vaccination against tuberculosis may indeed be small in most parts of this continent during times of peace and prosperity, much smaller than it is in the countries where tuberculosis is a more common disease. That need may, however, be correspondingly greater here as an insurance against the possible widespread outbreak of tuberculosis in times of war and calamity.

The protective vaccination against tuberculosis with all of its drawbacks and shortcomings may well prove to be an important measure in the whole picture of our preparedness for an emergency.

Summary

There was a small epidemic of 40 cases of tuberculosis in a Canadian village during a period of two years.

The measures taken in order to investigate and control this outbreak included a roentgenographic survey (99.2 per cent of those more than five years old were examined), a tuberculin survey, BCG vaccination of the negative reactors, follow-up examinations of those with positive reactions, and study of individual cases of tuberculosis. The adolescents and young adults constituted the segment of the population chiefly affected; the morbidity rate was 5.5 per cent in the age

group of fifteen through twenty-four years, and the tuberculin survey showed a marked rise in the percentage of positive reactors in this group.

The probable main sources of infection were among the young people themselves, and a small restaurant provided the most likely setting for its spread. The disease in the majority of these 40 patients was a direct result of recently acquired primary tuberculous infection.

Small epidemics of tuberculosis are becoming more frequent; they appear to arise among groups with no acquired resistance when potent sources of infection are introduced into an environment suitable for the spread of infection. These small epidemics may be a warning signal that the policy of withholding protective vaccination against tuberculosis in communities composed predominantly of negative reactors may have grave consequences in times of war or other calamity.

Sumario

Pequeña Epidemia de Tuberculosis

En una aldea canadiense hubo una pequeña epidemia de 40 casos de tuberculosis durante un período de dos años.

Las providencias tomadas a fin de investigar y dominar este brote comprendieron una encuesta radiográfica (se examinó a 99.2 por ciento de las personas de más de cinco años de edad), un estudio con tuberculina, vacunación con BCG de los sujetos negativos, exámenes subsiguientes de los reactores positivos y estudio de casos individuales de tuberculosis. Los adolescentes y los jóvenes constituyeron el segmento de la población principalmente afectado. El índice de morbidad fué de 5.5 por ciento en el grupo de quince a veinticuatro años de edad y la pesquisa con tuberculina reveló un aumento notable en el porcentaje de reactores positivos en este grupo.

Los principales focos probables de infección fueron los jóvenes mismos y un pequeño restaurante sirvió de centro más probable para la difusión. En la mayoría de estos 40 enfermos, la enfermedad fué consecuencia directa de la recién adquirida infección tuberculosa primaria.

Las pequeñas epidemias de tuberculosis se van volviendo más frecuentes; parecen surgir en grupos sin resistencia adquirida al introducirse focos potentes de infección en un ambiente apropiado para la propagación. Esas pequeñas epidemias tal vez sean un aviso de que la política de no suministrar vacunación protectora contra la tuberculosis en las colectividades compuestas predominantemente de sujetos negativos puede acarrear graves consecuencias en tiempos de guerra o de otra calamidad.

Resume

Une petite épidémie de tuberculose

Il s'est produit une petite épidémie de 40 cas de tuberculose dans un village du Canada au cours d'une période de deux années.

Les mesures prises pour l'investigation et le contrôle de cette épidémie comportaient le dépistage radiologique (99,2% des individus âgés de plus de 5 ans furent examinés), une enquête sur les résultats des tests à la tuberculine, la vaccination au B.C.G. des sujets à réaction négative, la surveillance des cas positifs et l'étude des cas de tuberculose individuels. Les adolescents et les jeunes adultes constituaient le segment de la population le plus affecté; le taux de morbidité était de 5,5% dans le groupe de quinze à vingt-quatre ans inclusivement. Dans ce groupe, l'enquête sur les tuberculin-tests révélait une élévation marquée du pourcentage des sujets ayant une réaction positive.

Les sources principales de l'infection résidaient vraisemblablement dans le groupe lui-

même et un petit restaurant a sans doute été le siège de la contamination. La majorité des cas de tuberculose, chez ces 40 malades, était le résultat direct d'une infection tuberculeuse primaire récente.

Les petites épidémie de tuberculose deviennent plus fréquentes, elles semblent surgir parmi des groupes dépourvus de résistance acquise lorsque des sources actives de contagion sont introduites dans un milieu favorable à la dissémination de l'infection. Ces petites épidémies peuvent être un signal d'alarme, en indiquant que la politique d'abstention à l'égard de la vaccination contre la tuberculose, dans les collectivités composées principalement de sujets anergiques, pourrait avoir des conséquences graves en période de guerre ou autre catastrophe.

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REFERENCES

(1) Mass B.C.G. Vaccination Campaign, UNICEF, Paris, 1953.
(2) Grzybowski, S.: Tuberculous infection in the population of Ontario, Canad. M. A. J. (in press).
(3) Wallgren, A.: The time table of tuberculosis, Tubercle, 1948, *29*, 245.
(4) Bentley, F. J., Grzybowski, S., and Benjamin, B.: Tuberculosis in Childhood and Adolescence, N. A. P. T., London, 1954.
(5) Shaw, J. B., and Wynn-Williams, N.: Infectivity of pulmonary tuberculosis in relation to sputum status, Am. Rev. Tuberc., 1954, *69*, 724.
(6) Bluhm, L.: Is there any risk of infection from gastric lavage positives, Acta tuberc. Scandinav., 1947, *21*, 70.
(7) Van Zwanenbert: Discussion at the meeting of the British Tuberculosis Association, Tubercle, 1955, *36*, 107.
(8) Ferguson, R. G.: Studies in Tuberculosis, University of Toronto Press, 1955.
(9) Waring, J. J.: The significance of small epidemics of tuberculosis, Dis. of Chest, 1955, *28*, 170.
(10) Maddison, G. E.: Epidemic tuberculosis, Medical Papers of Canadian Tuberculosis Association, 1952, 5.
(11) Smith, G. M., MacLellan, M., and Hiltz, J. E.: Pulmonary tuberculosis in a rural school and community: A follow-up study, Medical Papers of Canadian Tuberculosis Association, 1954, 5.
(12) Hyge, T.: Epidemic of tuberculosis in a state school, Acta tuberc. Scandinav., 1947, *21*, 1.
(13) Provincial Survey Committee of the Canadian Tuberculosis Association: Report of an Ontario Survey of School and Pre-school Children, Ontario Department of Health, 1925.
(14) Brink, G. C., Brown, M. H., and Gray, K. G.: Tuberculous infection in 2,492 persons, Canad. J. Pub. Health, 1933, *24*, 471.
(15) Myers, J. A.: Importance of tuberculin testing in school children: A twenty-year study, J. A. M. A., 1955, *195*, 185.
(16) Anderson, A. W., Grenville-Mathers, R., and Trenchard, H. J.: The prevention of tuberculosis in young people, Lancet, 1951, *1*, 1116.
(17) Price, D.: The need for B.C.G. vaccination in infants, Tubercle, 1949, *30*, 11.

SENSITIVITY TO TUBERCULIN AMONG STUDENTS AT THE UNIVERSITY OF MINNESOTA[1, 2]

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INTRODUCTION

In 1928 the tuberculin test was introduced as a routine part of the physical examination given entering students at the University of Minnesota (1). In that year it was found that 33 per cent reacted to tuberculin. The testing of each freshman class thereafter revealed a gradual annual decrease in the proportion of reactors, which, in 1945, had reached the low level of 6.4 per cent. In 1946, however, 19.3 per cent of entering students reacted to tuberculin (2). This was the first time in eighteen years that the proportion of reactors among entering students had failed to show an annual decrease and, since the rise was so precipitous, considerable concern was felt by those who had long worked to solve the tuberculosis problem.

While various possible causes of this phenomenon were considered, the outstanding fact was that in this year for the first time relatively large numbers of students had recently been discharged from military service. These students were, on the average, several years older than the typical entering freshman of previous years. This in itself might explain why they exhibited a higher reactor rate than that previously observed. In addition, there was the possibility that in the course of their military service, particularly while overseas, these young men had been subjected to a particularly great risk of contact with cases of contagious tuberculosis and, in consequence, many had thus acquired primary infections.

Accordingly, in 1947–1948, with the assistance of the Veterans Administration, a testing program was designed to elucidate the points at issue. It was decided, in so far as possible, to administer a tuberculin test to each male student at the University and to analyze reactor status in conjunction with the results of roentgenographic examination, residence and contact history, and details of military service. At the suggestion of Dr. Carroll E. Palmer of the United States Public Health Service, a program of testing with histoplasmin, coccidioidin, and blastomycin was carried out as part of this investigation. The result of the fungal antigen studies has been reported (3) and will not be further alluded to here except to note that no cross-reactivity whatever was noted between tuberculin and the fungal antigens.

[1] From the Students' Health Service, School of Public Health, and the Department of Internal Medicine, University of Minnesota, Minneapolis, Minnesota; and the Follow-up Agency, Division of Medical Sciences, National Research Council, Washington, D. C.

[2] Supported, in part, by a contract from the Veterans Administration upon recommendation of the National Research Council's Committee on Veterans' Medical Problems; and, in part, by the H. Longstreet Taylor Foundation, established by the Minnesota Tuberculosis and Health Association.

Plan of Investigation

A survey was made of 13,386 male students at the University of Minnesota, almost 99 per cent of whom were white. Since about 16,600 such students were enrolled at the University during the period of the survey, approximately 80 per cent of those eligible for study were actually examined.

The testing program was not compulsory for all students and, while repeated attempts were made to obtain the cooperation of those who did not reply to the first request, 19.5 per cent of the eligible men failed to report despite several solicitations. An attempt was made to obtain certain information by mail questionnaire from the 3,238 men who failed to report and it succeeded in 557 instances; for the remaining 2,681 men, only age, college, class, and veteran status were available through the cooperation of the Registrar's office.

Examination of these characteristics of the noncooperative students revealed that they were by no means randomly distributed through the student body. For example, only 6 per cent of students nineteen years of age or less failed to report, while the corresponding percentages in the other age groups were: twenty through twenty-four, 19 per cent; twenty-five through twenty-nine, 22 per cent; thirty and older, 25 per cent. Men registered at different colleges varied widely as to the probability of reporting for the study: For the schools of Agriculture, Forestry, Home Economics, Veterinary Medicine, Dentistry, Medicine, Education, and the General College, fewer than 5 per cent failed to cooperate; in the Adult Special category, 11 per cent failed; 19 per cent of the students at the Technical School did not report. The proportions failing to cooperate at the other schools were: Pharmacy, 21 per cent; Business, 25 per cent; Liberal Arts, 25 per cent; Law, 33 per cent; and Graduate School, 42 per cent.

Interestingly enough, however, veterans did not differ from nonveterans with respect to the probability of noncooperation: The percentages were 20.6 and 20.5, respectively.

The chief reason for the large variation between students at the various schools is to be found in the nonuniform University regulations regarding physical examinations. Yearly examination is required of all students at the schools of Medicine and Dentistry, while in the School of Education and the General College examination is required at entrance and graduation. Since the General College is a two-year school, this too is equivalent to yearly examination. At other schools, examination is required only on entrance. In a word, then, high proportions of response were obtained only at those schools where University regulations compelled attendance, while the level of cooperation from students not required to report to the Health Service was low.

What practical effect this selection had on the results is impossible to say. That at least a few men declined tuberculin tests because they thought themselves to be reactors cannot be disputed. However, it is believed that, in so far as the majority of the men were concerned, the factors which determined whether or not men would report (when it was not compelled by regulation) had more to do with the personality of the individual, how pressed for time he was, et cetera, than with knowledge of tuberculin sensitivity or exposure. In any event, the fact that the response was almost identical for veterans and for nonveterans gives reason to believe that, in so far as this main comparison is concerned, the results probably are not seriously affected by bias.

A single dose of tuberculin was employed: 0.1 mg. of original tuberculin. Almost all readings were made at seventy-two hours (87.8 per cent), but a few students reported at forty-eight hours, ninety-six hours, and even one hundred and twenty hours. Fortunately, only about 3 per cent of the reactions were read at one hundred and twenty hours, for at this time there was evidence of some diminution of average diameter of induration.

Tuberculin test results were not obtained for all of the 13,386 men surveyed. Only 11,487 were read, while 1,899 (a little more than 14 per cent) were not. In some cases, at least, the tuberculin test was refused because of a known previous positive reaction; in other instances, negative tuberculin tests had been recorded in the recent past. The records of previous tuberculin tests were utilized for these persons, correcting age to that at the test recorded rather than age at the survey, when necessary. Previous tuberculin tests made at

the University were available for 928 of the 1,899, so that tuberculin status was known, either at the time of the survey or for some relatively recent date, for 12,415 men and unknown for 971 (7.3 per cent) of the men surveyed. The most important sources of possible bias would seem to reside in this group of 971 men.

Actually, the 971 men in question fell into two groups: 483 for whom neither the tuberculin nor the triple fungal antigens were read, and 452 for whom the fungal antigen results were read but not the tuberculin results. On comparing these two subgroups with the whole group of 12,415 for whom tuberculin tests were done, it was found that the former subgroup (who failed to report for reading of results) were quite like the 12,415 with respect to roentgenographic results and history of "lung disease." However, the 452 men who refused the tuberculin test specifically were clearly special: Only 92.7 per cent of them had negative roentgenograms as compared with 97.2 per cent of the men for whom tuberculin results were known; while 11.1 per cent claimed a history of tuberculosis, contrasted with only 0.5 per cent of the 12,415. Higher proportions claimed histories of pleurisy (2.9 per cent *versus* 0.8 per cent) and bronchitis (2.4 per cent *versus* 1.5 per cent). It must be concluded that an unknown but perhaps large fraction of the 452 men declined the tuberculin test because they knew or thought they knew themselves to be reactors. If these men were all reactors, and had been included in the group of men tested, the over-all reactor rate would have been increased by approximately 15 per cent of its value, since actually 2,895 tuberculin reactors were identified. However, while it seems probable from the indirect evidence cited that this group of men contained quite a high proportion of reactors, it seems unlikely that every one of them was a reactor. In fact, of men who were tested who claimed a history of tuberculosis, only 71.4 per cent were reactors (table 5). It may be concluded that probably the tuberculin reactor rates were too small by not more than 10 per cent of their value. This is the effect which would be produced if two-thirds of the group were reactors.

Observations

The over-all results of the tuberculin testing program are shown in table 1. A positive reaction is defined as an area of induration measuring at least 5 mm. in greatest transverse diameter. This definition will be adhered to throughout except where reactions of smaller induration are explicitly mentioned. In the subsequent discussion no attempt will be made generally to distinguish between the group previously tested and the group tested as a part of the survey.

The reactor rates in terms of age and veteran status are shown in table 2 and figure 1. Remarkably enough, while the veterans had higher rates than nonveterans at the ages of twenty-five and younger, of those older it was the nonveterans who were more frequently reactors. Although the over-all reactor rates for veterans and nonveterans were quite different (27.0 per cent and 13.4 per cent), the greater part of the difference is attributable to differences in the age distributions of the two subsamples. This is shown by the fact that, if the rates in the two groups are standardized (by the so-called "indirect method" (4)) to the age

TABLE 1

Results of Tuberculin Tests

	Total Survey	Tested in Survey	Previously Tested at University of Minnesota
Number of men	12,415	11,487	928
Number of reactors	2,895	2,548	347
Per cent of reactors	23.3	22.2	37.4

TABLE 2

RESULTS OF TUBERCULIN TESTS BY AGE AND VETERAN STATUS

Age in Years	All Men		Veterans		Nonveterans	
	Number Tested	Per Cent of Reactors	Number Tested	Per Cent of Reactors	Number Tested	Per Cent of Reactors
Total.....	12,415	23.3	9,062	27.0	3,353	13.4
18 and less...	741	7.6	8	*	733	7.4
19.....	1,030	8.9	40	7.5	990	9.0
20.....	1,085	13.3	328	20.4	757	10.2
21.....	1,238	19.1	928	20.5	310	14.8
22.....	1,304	19.9	1,197	20.4	107	15.0
23.....	1,316	23.1	1,224	23.1	92	22.8
24.....	1,240	25.2	1,177	25.7	63	15.9
25.....	1,061	27.7	1,016	28.0	45	22.2
26.....	857	28.7	814	27.6	43	48.8
27.....	645	30.7	617	30.3	28	39.3
28.....	511	32.7	483	31.9	28	46.4
29.....	350	38.6	327	38.2	23	43.5
30-34.....	702	42.0	640	41.4	62	48.4
35 and older..	183	54.1	132	50.0	51	64.7
Not stated...	152	37.5	131	37.4	21	38.1

* There were two reactors in this small group.

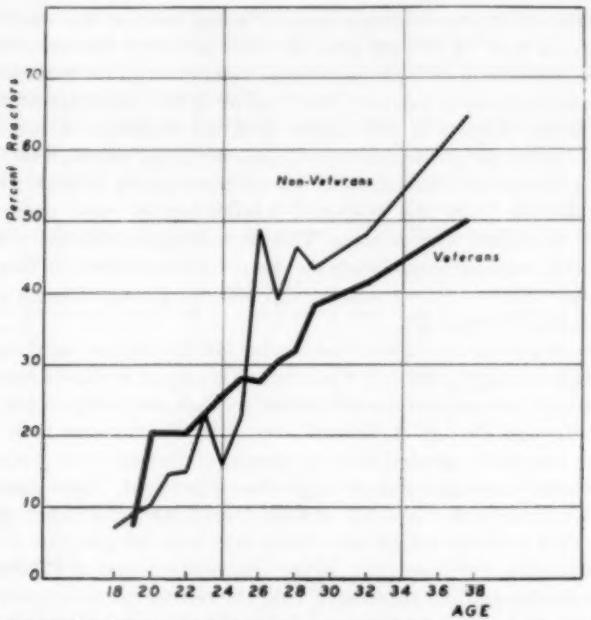

FIG. 1. Per cent of tuberculin reactors by age for veterans and nonveterans (data from table 2).

distribution of the total sample, standardized rates are obtained of 23.6 per cent for veterans and 22.0 per cent for nonveterans.

This closeness of the age-standardized rates is perhaps less interesting than the rather large difference between the slopes of the curves in figure 1 which show the rates in terms of age for the two groups of men. The (indirect) standardized reactor rate for nonveterans aged twenty-six and older was 44.7 per cent, while for veterans it was only 34.2 per cent. This difference is surprisingly large and not entirely explicable. Undoubtedly, the fact that most men with a previous history of tuberculosis were ineligible for military service would account for some fraction of the differential. Three and six-tenths per cent of the total of more than 4,500,000 men who had been rejected for military service up to April 1, 1945, had been rejected because of tuberculosis (5). Moreover, this figure is probably an underestimate of the proportion of rejected men who had had tuberculosis. Men who had tuberculosis before entering college would have been delayed in their scholastic careers and such men would, therefore, tend to fall into the group of nonveterans, aged twenty-six and older.

Among veterans, no significant differences in reactor rates were found between Army and Navy veterans, and men who served either in the Army or Navy; or between men who did or did not have overseas service. While some small differences were seen in the crude reactor rates, standardization for age reduced the differences to values not exceeding those which could be attributed to chance in a random sampling procedure.

One subgroup of veterans characterized by a high reactor rate was that of former prisoners of war. Of 90 such men, 32 (35.6 per cent) reacted to tuberculin, compared with a rate of 28.5 per cent for all veterans with overseas service. Perhaps the most surprising aspect of this finding is the smallness of the rate in former prisoners, since it is well known that the incidence of tuberculosis in prisoner-of-war camps, particularly the Japanese camps, was high (6).

In view of the rather small excess in reactor rates among ex-prisoners, it is not surprising that no particular places of foreign service were associated with significantly increased reactor rates. This is consistent with the results of an investigation of men discharged from the Army for tuberculosis in World War II, which demonstrated no strong relationship between place of foreign service and the tuberculosis incidence (7).

It may be seen from table 2 that the reactor rate in students aged eighteen and nineteen years was approximately 8 per cent. This figure would be produced by a constant rate of conversion of about 0.46 per cent per year for men not previously converted. Beyond the age of nineteen, however, the increase with age in the reactor rate was much greater, and the annual conversion rate (for men previously uninfected) averaged slightly more than 3 per cent. These figures may be compared with the finding in a test of more than 6,000 Minneapolis *grade school children* in 1944 that the annual conversion rate over the previous six years had been about one-third of 1 per cent. In the University School of Education it was found that for the graduating class of 1948 the reactor rate increased from 24.8 on entrance to 28.5 on graduation, or 3.7 points in four years. Among students of

medicine, during the four years they were on the campus the proportion increased only 1.5 percentage points (33.8 on entrance to 35.3 on graduation) for the class graduating in 1944 and 6.6 points (31.7 to 38.3) for the class of 1945. Thus the average for all of this material reveals a net conversion rate of approximately 0.9 per cent annually for students at the University.

Given the rather steep rise of the reactor rate with age, it is not surprising to find that the rate varied markedly with class (table 3). Age standardization to the entire student body has the effect of removing almost all trace of movement, however. There is no evidence that, apart from age, residence at the University is associated with particularly high (or low) conversion rates.

The reactor rates for students at the various schools are shown in table 4. Here, again, rates standardized (by the indirect method) to the age distribution of the entire study sample have been calculated in order to eliminate the confounding which results from the fact that age distributions varied at the different schools. It will be noticed that the variability in rates between schools is markedly reduced by the standardization procedure; in fact, the only standardized rate which differs significantly from the average is that pertaining to the School of Agriculture and related schools. This rate is significantly lower than the general rate (17.8 *versus* 23.3), being only about three-quarters of the general rate. The reason for this difference is almost certainly to be found in the differing residence histories of students in the agricultural and related schools as opposed to other schools. While 38.9 per cent of the agricultural students characterized their longest residence as "farm," only 8.8 per cent of students at other schools had farm backgrounds. As may be seen below, the former farm dwellers in the student body were characterized by low reactor rates as opposed to students of urban or mixed urban and rural backgrounds.

It is perhaps surprising, and certainly gratifying, that students at the Medical School were not characterized by a reactor rate abnormally high for their age; the age standardized rate was 26.7—some 3.4 points higher than the general average of 23.3—but this difference is not large enough to achieve statistical significance for the sample sizes in question. While this observation does not, of

TABLE 3

REACTOR RATES BY CLASS: CRUDE AND AGE-STANDARDIZED

Class	Number Tested	Per Cent of Reactors	
		Crude	Age-Standardized
Total	12,415	23.3	23.3
Freshman	2,678	14.5	22.6
Sophomore	2,496	19.8	22.0
Junior	2,673	22.3	21.2
Senior	3,654	29.2	24.7
Graduate students	766	39.7	26.5
Unclassified	128	32.0	23.9
Not stated	20	25.0	20.0

course, demonstrate that there was no increased risk whatever for this group, it seems plain that the medical students were exposed to no increased risk of substantial magnitude.

Of the 12,415 men for whom tuberculin test results were available, 10,239 (82.5 per cent) had lived longer in Minnesota than in any other state. The only states other than Minnesota which were claimed by as many as 100 men as states of longest residence were Wisconsin, 374 (3.0 per cent); North Dakota, 269 (2.2 per cent); Iowa, 250 (2.0 per cent); South Dakota, 204 (1.6 per cent); and Illinois, 159 (1.3 per cent). Together these last five states accounted for 1,256 men, or 57.7 per cent of those who were not Minnesota residents. This material is, therefore, ill suited to a study of the geographic variation in tuberculin. Nevertheless, it is apparent that Minnesota residents were somewhat favored: The 10,239 Minnesota residents had a reactor rate of 21.7 while the remaining 2,176 men had a rate of 30.8.

Minnesota students whose longest residences were on farms had a significantly lower reactor rate than those whose residences were non-farm—16.7 per cent of 281 reacted in the first group *versus* 21.8 per cent of 8,440 in the second group. The 1,367 men with mixed farm and non-farm backgrounds had a rate of 21.9—almost identical with that of the non-farm residents.

Only 179 (1.4 per cent) of the students were non-white, but the proportion of tuberculin reactors in this group was more than double the rate in white students: 95 of the 179 reacted, a rate of 53.1 per cent, contrasted with a rate of 22.9 per cent in the 12,236 white students.

Provocative data will be found in table 5, which relates the history of lung disease claimed by the students to their reactor status to tuberculin. It is rather surprising that less than three-quarters (71.4 per cent) of the men who thought

TABLE 4

REACTOR RATES BY SCHOOL: CRUDE AND AGE-STANDARDIZED

School	Number Tested	Per Cent of Reactors	
		Crude	Age-Standardized
Total	12,415	23.3	23.3
Agriculture, Forestry, Home Economics, Veterinary Medicine	496	17.9	17.8
Business	1,087	27.0	23.8
Medical, Dental, Occupational Therapy, Physical Therapy, Public Health	506	32.8	26.7
Education	568	28.5	23.7
General	854	18.5	25.5
Graduate	573	39.4	26.5
Institute of Technology	3,623	22.4	22.4
Law	385	22.1	19.8
Pharmacy	198	23.2	22.9
Liberal Arts, University College	3,874	20.7	23.4
Adult special or not stated	251	22.3	19.9

they had had tuberculosis reacted to tuberculin. This demonstrates again, if further demonstration is needed, how often diagnoses of tuberculosis can be in error when made solely on roentgenographic evidence. The elevated reactor rate in students with a history of pleurisy is strong evidence that much of the pleurisy was tuberculous in its etiology; but the fact that fewer than half of the men who had a history of pleurisy had a positive tuberculin reaction shows that most of these pleurisies were nontuberculous. Finally, it is clear that not more than a tiny factor of morbid episodes described as pneumonia, bronchitis, influenza, or asthma could have represented tuberculosis, since men who gave histories of these conditions reacted to tuberculin no more frequently than did men without such histories.

In table 6 the data with respect to the history of contacts with tuberculosis claimed by the students are given. It will be noted that 21.2 per cent—more than

TABLE 5

REACTOR RATES BY HISTORY OF LUNG DISEASE

Lung Disease in History	Number Tested	Number of Reactors	Per Cent of Reactors
Total	12,415	2,895	23.3
None	11,064	2,562	23.2
Tuberculosis	56	40	71.4
Pleurisy	100	44	44.0
Pneumonia	810	169	20.9
Bronchitis	188	33	17.6
Influenza	63	14	22.2
Asthma	96	21	21.9
Other	38	12	31.6

TABLE 6

REACTOR RATES BY TUBERCULOSIS CONTACTS

Tuberculosis Contacts	All Men		Veterans		Nonveterans	
	Number Tested	Per Cent of Reactors	Number Tested	Per Cent of Reactors	Number Tested	Per Cent of Reactors
Total	12,415	23.3	9,062	27.0	3,353	13.4
No known contact	10,226	21.2	7,309	24.8	2,917	12.0
Non-household contact	1,641	30.2	1,308	33.0	333	19.5
Immediate family of other household contact	548	42.9	445	44.9	103	35.0
At least 2 contacts*	194	50.5	155	51.6	39	46.2
Non-household contact in Service			336	35.1		
Non-household contact, not in Service	1,305	29.0	972	32.2	333	19.5
Care of tuberculosis patients, in Service			87	47.1		

* Claimed tuberculosis contacts in at least two of the three categories:
(*a*) Member of immediate family while living in same household
(*b*) Other person living in same household
(*c*) Other person not living in the same household

one in 5 persons—of those who denied any contact nevertheless reacted to tuberculin. On the other hand, of 548 men who claimed contact with a patient with tuberculosis as a member of the immediate household, only 42.9 per cent reacted. Clearly, then, no great reliance can be placed on the unsupported statement of the subject of whether or not he has had an effective tuberculosis contact, i.e., a contact which established at least that degree of infection necessary to initiate an allergic response to tuberculin.

There are several reasons why this is true: First, many diagnoses are erroneous. As already seen, almost 30 per cent of the students who believed that they themselves had had tuberculosis were probably in error, since they failed to react to tuberculin. Second, many contact cases, which are really tuberculous, are not infectious. Finally, many infectious cases of tuberculosis are undiagnosed and unsuspected.

Despite these obvious weaknesses, it remains true that some correlation exists between statements of contact and the existence of allergy to tuberculin. In the entire group tested, the reactor rate was about twice as great in men who claimed a household contact as in those who knew of none (42.9 per cent *versus* 21.2 per cent). The men who claimed a contact with a person not in the same household manifested a rate intermediate between the two, and those who asserted at least two contacts had the highest rate, 50.5.

Among veterans, contacts in service were not more effective than equivalent (not in the same household) contacts outside of service. The small difference in rates (35.1 per cent *versus* 32.2 per cent) is not statistically significant.

The 89 veterans who asserted that they had had the care of tuberculous patients as one of their duties in service had a significantly higher reactor rate (47.1 per cent) than the balance of the 1,308 veterans who claimed a non-household contact (1,221 men, 31.9 per cent). The value of chi-square for this difference is 7.80, $p < 0.01$.

Finally, the difference in level between veterans and nonveterans which persists through all levels of contact probably reflects the age differences between the two groups of men, which were considerable. For any degree of contact, the veterans, on the average, have been exposed not only to the contact (in which they may be comparable with the nonveterans) but also to several additional years of random mixing in society, which, the age curves reveal, is accompanied by a steady increase in the proportion of reactors.

There were 256 students who presented some manifestation of chest abnormality by roentgenographic examination and for whom tuberculin reactor status was known. Of the 74 in whom only calcification was seen on the roentgenograms, only 25.7 per cent reacted to tuberculin (table 7). Tuberculosis is but a single one of many conditions which cause calcium deposition in the lungs, hilar lymph nodes, and pleura. It is not unusual to find groups of persons in whom the preponderance of calcium deposits is of nontuberculous etiology.

In the present study, of 154 persons classified as showing abnormalities other than calcification on a chest roentgenogram, 33.1 per cent reacted to tuberculin. Obviously, in making films of the chest of large numbers of persons, abnormalities of various etiologies came to light. No case of clinical pulmonary tuberculosis was

found during this survey, inasmuch as all students had been carefully screened for this disease on admission to school.

Evidence suggesting existence of *nonspecific* reaction to tuberculin was adduced by Palmer and Peterson (8) and by Palmer, Ferebee, and Peterson in 1950 (9). Taylor reviewed Palmer and Peterson's data from a somewhat different methodologic point of view (10). Nevertheless, these writers all agreed in concluding that the data were inconsistent with the simple model which assumes merely that subjects who are tested are either "true positives" or "true negatives." In such a simple model, each member of a class has a certain different probability of reacting equivocally to tuberculin, but members of the first group never give any reaction and members of the second group never give a definite reaction.

Although the present observations on the results of the tuberculin test did not include as an aid in classifying reactions the qualitative description of the induration employed by Palmer and associates, varying degrees of reaction were noted simply in terms of the diameter of induration.

The data on seventy-two-hour readings are shown in table 8. The various readings have been grouped into sets centered on the multiples of 5 because of a clear

TABLE 7

REACTOR RATES BY ROENTGENOGRAPHIC INTERPRETATION

Roentgenographic Interpretation	Number Tested	Per Cent of Reactors
Total	12,415	23.3
Negative	12,070	23.2
Calcification only	74	25.7
Other abnormalities than calcification	154	33.1
Calcification and other abnormalities	28	42.9
Roentgenogram not obtained	89	20.2

TABLE 8

DIAMETER OF TUBERCULIN TEST INDURATION, 72-HOUR READINGS

Diameter of Induration	Number of Readings
Total	10,085
No reaction	7,664
Erythema without induration or induration not more than 2 mm.	144
3–7 mm.	75
8–12 mm.	364
13–17 mm.	272
18–22 mm.	602
23–27 mm.	492
28–32 mm.	313
33–37 mm.	95
38 mm. or more	55
Positive reaction, diameter not stated	9

tendency for such concentration in the measurements. It will be recalled that the induration reported was the greatest transverse diameter. The most noteworthy feature of the data in table 8 is the decline from 364 persons with a diameter of induration 8–12 mm. to 272 in the class 13–17 mm., followed by a steep rise to 602 in the 18–22 mm. group. The bimodality of this distribution was too striking to be dismissed as a vagary of sampling.

The distribution of size of induration was examined in relation to many different factors in an attempt to discover the reason for this peculiarity. The most striking finding may be seen in table 9, namely, the relationship between the diameter of induration and the results of previous tuberculin tests, when these were present in the Student Health Service records. The impressive feature of this table is the disappearance of bimodality from the distribution which is limited to men for whom a previous positive tuberculin test was recorded. On the other hand, among men previously known to have been negative, the height of the peak at approximately 10 mm. is emphasized. Among men with previously positive tests, the modal diameter class is that centered at 25 mm., while among previous "negatives" the classes at 10 mm. and 20 mm. are almost identical, and there is a sharp fall-off to 25 mm. Finally, the distribution of diameters of induration for men for whom no previous test was recorded is quite similar to the entire series. It is worth noting, too, that if the distributions for those previously negative are combined with those previously positive, a distribution resembling the entire series is again obtained.

The difference between the distribution for men previously negative and previously positive was highly significant: $\chi^2 = 114.6$ on 5 degrees of freedom (combining each of the first two and last two induration classes) so $p < 0.0001$.

In table 10 are shown the distributions which result when the men are classified not only by the positive or negative character of previous tuberculin tests but also according to the time since the earliest positive or latest negative previous

TABLE 9

DIAMETER OF INDURATION OF TUBERCULIN TEST, 72-HOUR READINGS, IN RELATION TO RESULTS OF PREVIOUS TUBERCULIN TESTS

Diameter of Induration	All 72-Hour Readings		Result of Previous Tuberculin Test					
			Positive		Negative		Not Available	
	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent
Total	2,268	99.9	485	100.1	481	100.0	1,302	100.0
5–7 mm.	75	3.3	13	2.7	29	6.0	33	2.5
8–12 mm.	364	16.0	41	8.5	126	26.2	197	15.1
13–17 mm.	272	12.0	43	8.9	73	15.2	156	12.0
18–22 mm.	602	26.5	112	23.1	127	26.4	363	27.9
23–27 mm.	492	21.7	138	28.5	62	12.9	292	22.4
28–32 mm.	313	13.8	87	17.9	43	8.9	183	14.1
33–37 mm.	95	4.2	36	7.4	11	2.3	48	3.7
38 or more	55	2.4	15	3.1	10	2.1	30	2.3

test was recorded. Among men who were previously positive, the time factor was of no significance: $\chi^2 = 13.90$ on 10 degrees of freedom $0.20 > p > 0.10$. (The first two and last two induration classes again were each combined in order to perform the test.) However, among men previously negative, the time factor was highly significant: Here $\chi^2 = 43.37$, 10 degrees of freedom, $p < 0.001$.

The distributions are shown graphically in figure 2: It may be seen from the figure or from table 10 that, as the length of time since the last previous negative test increases, the distribution of diameters of induration approaches more nearly the shape of the distribution for men who were previously positive.

Remarkably enough, no other characteristic which was examined seemed to be related (among men previously negative) to the diameter of induration. While the history of lung disease, tuberculosis contacts, roentgenographic interpretation, and age were all related to size of induration on the whole group of reactors, they were not so within the subgroup of men previously negative. These characteristics, of course, were strongly related to the chance of a previous test's having been positive.

TABLE 10

DIAMETER OF INDURATION OF TUBERCULIN TEST, 72-HOUR READINGS, IN RELATION TO RESULTS OF AND TIME SINCE PREVIOUS TUBERCULIN TESTS

Diameter of Induration	Previous Positive Test			Previous Negative Test		
	Time Since Earliest Recorded Positive			Time Since Last Recorded Negative		
	Not More Than 1 Year	2 Years	3 Years or More	Not More Than 1 Year	2 Years	3 Years or More
	Number of Men					
Total	176	169	140	175	126	179
5–7 mm.	2	1	10	14	6	9
8–12 mm.	18	11	12	57	34	35
13–17 mm.	22	11	10	31	19	23
18–22 mm.	37	46	29	51	37	39
23–27 mm.	49	53	36	6	18	38
28–32 mm.	27	31	29	9	10	23
33–37 mm.	16	10	10	4	—	7
38 or more	5	6	4	3	2	5
	Per Cent					
Total	99.8	100.0	99.9	99.9	100.1	99.9
5–7 mm.	1.1	0.6	7.1	8.0	4.8	5.0
8–12 mm.	10.2	6.5	8.6	32.6	27.0	19.6
13–17 mm.	12.5	6.5	7.1	17.7	15.1	12.8
18–22 mm.	21.0	27.2	20.7	29.1	29.4	21.8
23–27 mm.	27.8	31.4	25.7	3.4	14.3	21.2
28–32 mm.	15.3	18.3	20.7	5.1	7.9	12.8
33–37 mm.	9.1	5.9	7.1	2.3	—	3.9
38 or more	2.8	3.6	2.9	1.7	1.6	2.8

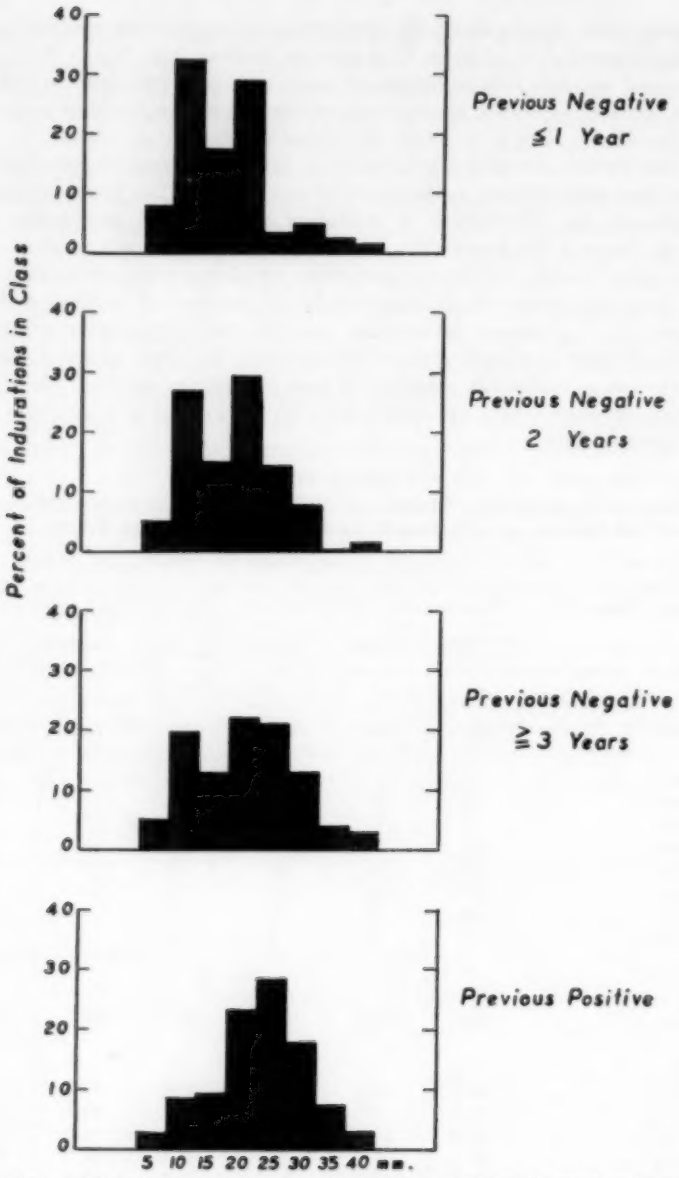

FIG. 2. Per cent distributions of diameter of induration of tuberculin reactors for previous reactors and previous nonreactors, the latter categorized by time since last test.

In particular, character of residence as rural or urban seemed to have no influence on the diameter of induration, again among men previously negative. The number of such men (481) was too small to permit analysis in relation to location of residence, especially since the great majority came from the Twin Cities.

Discussion

Since 1928, testing entering students with tuberculin has provided the best criterion of the magnitude of the tuberculosis problem among the students. The number of clinical cases developing among students paralleled the number of reactors, that is, as the reactor rate decreased, there was a corresponding decline in the incidence of clinical tuberculosis. Therefore, the relatively high incidence of reactors among entering students in 1946 was viewed with alarm. However, it was gratifying to find that at least a considerable part of the increased incidence was explainable on the basis of age. This had been observed over the years prior to World War II among groups of students who were definitely older than the average on admission, of whom those admitted to the School of Medicine represented the best example. It was especially pleasing to discover that the military services exercised such effective protective measures in parts of the world where contagious tuberculosis was still extremely prevalent among the citizenry.

Although the number of non-white students surveyed was small, the reactor rate was more than double that of the white students. This has been a common observation among the students from abroad, both before and since this survey was conducted. The percentage incidence of clinical disease among students from other countries with excessive reactor rates has been much higher than that among native American students.

Beginning in 1929, all entering students had fluoroscopic inspection of the chest. In 1946, photofluorograms were substituted for fluoroscopy. Since 1930, all students who reacted to tuberculin on entrance have had regular chest roentgenograms and, thus, those who had evidence of clinical disease were rejected or kept under close surveillance. This screening procedure had been applied to all who were in school at the time of the present survey. This in large part accounts for the fact that no clinical pulmonary tuberculosis was found on the re-survey of these students. The abnormalities seen on roentgenograms in addition to calcification consisted mainly of such residuals as diaphragmatic adhesions.

Calcification in the lungs and regional lymph nodes may be caused by a number of conditions which cannot be differentiated by roentgenography. Moreover, it has been shown that roentgenograms during life reveal only about one-fourth of the cases in which calcium deposits are clearly in evidence at necropsy. Thus, failure to find evidence of calcium deposits in this study did not prove their absence.

Among those who gave histories of the diagnosis of tuberculosis in the past, only 71.4 per cent reacted to tuberculin during this survey. It is possible that, in some of these students, sensitivity to tuberculin had waned to such a level that it

was not elicited by 0.1 mg. of tuberculin. However, it seems likely that errors in diagnosis were more responsible. Too often diagnoses have been made from roentgenographic shadows, which are never pathognomonic, and specific evidence (including the tuberculin reaction and the recovery of tubercle bacilli) has not been sought. Failure to require specific evidence in the diagnosis of tuberculosis has resulted in the classification of tuberculosis in large numbers of persons with such conditions as sarcoidosis, neoplastic disease, pneumonia, nonspecific fibroses, and fungal infections—especially histoplasmosis.

A history of exposure to tuberculosis is frequently unreliable. In this survey, one in 5 of the tuberculin reactors had no knowledge of contact with a contagious case of tuberculosis. In some groups of tuberculin reactors, a higher percentage has reported unawareness of contact with tuberculous patients. This is not surprising, inasmuch as most persons with chronic pulmonary tuberculosis pass through a long silent period, during a part of which the disease is contagious. Others do not have diagnoses made until after symptoms appear. On the other hand, there are many persons who think they have had contact with tuberculous patients, but the diagnoses in such cases have been in error, the contact has not been sufficient to result in invasions with tubercle bacilli, or their cutaneous sensitivity has waned below the level of the dose of tuberculin used in the present study. In fact, among 548 men who gave histories of contact in their immediate families or other persons in the same household, only about 43 per cent reacted to tuberculin.

Too often, pleurisy with or without effusion has been diagnosed as tuberculous without specific evidence. In fact, 56 per cent of students who gave histories of pleurisy did not react to tuberculin. In part, this may have been for the reasons presented above with reference to diminished sensitivity to tuberculoprotein, but most likely these pleurisies were not due to tuberculosis. In cases of pleurisy with undetermined etiology, biopsy has proved of such value that the number of cases classified as idiopathic should rapidly decrease.

For many years, the present writers have regarded an area of edema or induration or both that attained 5 mm. or more in diameter as a definite reaction to tuberculin. When skin changes under this level were present, they have been considered questionable.

Sensitivity to tuberculin has long been known to vary from time to time. Within a few days after an invasion with tubercle bacilli, sensitivity has been detected by administering the tuberculin test and later making microscopic observations of biopsy material from the skin. Apparently, during the succeeding weeks (three to seven) before the large characteristic tuberculin reaction can be elicited, sensitivity gradually increases. It is probable that some of the small reactions are due to developing sensitivity which has not reached the degree of producing a large reaction. For this reason, such small skin changes probably should always be reported as questionable and the test repeated a few weeks later.

There are other forms of acid-fast bacilli which may sensitize the tissues to a

slight degree and be responsible for mild reactions. In avian tubercle bacillus infections, tuberculin made from bacilli of the mammalian type does not always result in characteristic reactions. While a typical reaction may be elicited, mild to no skin changes occur. For this reason, in seeking infections with avian tubercle bacilli, tuberculin made from that type of organism should be employed. Although only approximately twenty-five well-documented cases of clinical tuberculosis caused by the avian tubercle bacillus in man have been reported, observations have been far too limited, and much more extensive and intensive studies are indicated. It may well be that some of the smaller and noncharacteristic reactions observed in this survey were due to infection with avian tubercle bacilli. Many students who participated in this survey have lived in areas where there has been a large volume of avian tuberculosis among both fowls and swine. Nonpathogenic acid-fast bacilli constitute another possibility inasmuch as they are so prevalent in some places.

Forty years ago veterinarians encountered a similar problem. Some animals did not present characteristic reactions to tuberculin while, in others, reactions were too slight to be interpreted as positive but just serious enough to be disturbing. Acid-fast soil bacilli were found responsible for a part of this mild degree of sensitivity to tuberculoprotein. Some of the reactor animals had small nodules in the skin of the lower extremities from which acid-fast soil bacilli could sometimes be obtained. Animals with such mild reactions were usually marked as "suspicious" and retested in two or three months, by which time they usually failed to react unless an extension of skin nodules had occurred. The sensitivity, therefore, appeared to be only of a transitory nature. Moreover, the skin lesions gradually resolved until there was scarcely a vestige left after three years.

Slight and noncharacteristic reactions to tuberculin may be observed as allergy wanes. Several writers have reported persons who presented characteristic reactions who later failed to react to tuberculin. This is presumed to be due to complete disappearance of tubercle bacilli in the body or to so little activity among them that not enough tuberculoprotein is being liberated to maintain allergy at a high enough level to be elicited by the first and sometimes even the second test dose of tuberculin. So few observations have been made in this field that little is known of the time required for allergy to wane to this level. It is thought that as allergy is waning, it may be restored to its former high level by reinfection with tubercle bacilli.

From the clinical point of view, allergy at low level before having attained its plateau in recent disease or while it is waning from long-standing, nonprogressive lesions may result in diagnostic errors in that during either of these periods it may not be elicited by the usual first test dose of tuberculin. Therefore, it may be overlooked if a larger amount of tuberculin is not administered. This is also true in some persons with acute, rapidly progressive disease and in those with chronic lesions approaching or in the terminal stage. Occasionally persons have been observed to react to only a large dose of tuberculin, but their lesions when resected or seen at necropsy were tuberculous.

The medical profession is seriously handicapped in determining the exact cause

of small and noncharacteristic tuberculin reactions because the opportunity is lacking in most cases to make a careful search promptly for the responsible organism. The veterinarian's advantage in this respect usually enables him to determine etiology accurately.

Because of the necessity of indirect approach, this subject is one that requires a tremendous amount of investigation. If people are to be treated for tuberculosis on the basis of recent tuberculin conversion, it would be unfortunate to administer long courses of treatment to those uninfected with pathogenic tubercle bacilli. On the other hand, it would be more unfortunate to consider a tuberculin reaction too mild or noncharacteristic or entirely lacking to a given dose of tuberculin and thus fail to diagnose tuberculous lesions which at the time or soon after may be contagious.

Summary

The incidence of tuberculin reactors among students entering the University of Minnesota increased from 6.4 per cent in 1945 to 19.3 per cent in 1946. A large number of World War II veterans entered in 1946, and this study was undertaken to determine whether the increased incidence of infection was due to excessive exposure in military service or to the fact that the veterans were several years older than the usual students.

Various factors, including tuberculosis contacts, history of previous lung disease, roentgenographic findings, and the diameter of the tuberculin reaction, were studied.

Of the 12,415 students tested, 23.3 per cent reacted to tuberculin. Of the total number, 9,062 were veterans, 27 per cent of whom reacted; and 3,353 were nonveterans, 13.4 per cent of whom reacted. With few exceptions, differences in age of veterans and nonveterans were adequate to account for the higher incidence of reactors among veterans.

No particular place of foreign service was associated with significantly increased tuberculin reactor rates.

During the war many veterans were stationed in parts of the world where tuberculosis was rife among civilians. Evidently, those in charge of the military services did excellent work in protecting servicemen from tubercle bacilli.

Sumario

Sensibilidad a la Tuberculina entre los Estudiantes de la Universidad de Minnesota

La incidencia de reactores a la tuberculina entre los estudiantes que ingresan en la Universidad de Minnesota aumentó de 6.4 por ciento en 1954 a 19.3 por ciento en 1946. Un número elevado de veteranos de la II Guerra Mundial ingresó en 1946, y se emprendió el estudio actual para determinar si la mayor incidencia de infección se debía a exposición excesiva en el servicio militar o a que los veteranos tenían algunos años más de edad que los estudiantes habituales.

Discútense varios puntos, incluso contactos con tuberculosis, historia de afección pulmonar anterior, hallazgos radiográficos y diámetro de la reacción a la tuberculina.

De los 12,415 estudiantes comprobados, 23.3 por ciento reaccionaron a la tuberculina.

Del total dado, 9,062 eran veteranos, reaccionando 27 por ciento de ellos, y 3,353 no lo eran, reaccionando 13.4 por ciento de éstos. Con pocas excepciones, las diferencias en edad entre los veteranos y los otros estudiantes bastaban para explicar la mayor incidencia de reacciones entre los primeros.

No se vinculó ningún sitio dado de servicio en el extranjero con los índices significativamente más altos de reactores a la tuberculina. Durante la guera, muchos veteranos habían estado acuartelados en partes del mundo donde la tuberculosis prevalecía en la población civil. Manifiestamente, los encargados de los servicios militares ejecutaron una labor excelente en lo tocante a proteger a los guerreros contra los bacilos tuberculosos.

Resume

Sensibilité à la tuberculine chez les étudiants de l'Université de Minnesota

L'incidence de la réaction à la tuberculine parmi les étudiants entrant à l'Université de Minnesota a augmenté de 6,4% en 1945 à 19,3% en 1946. Un grand nombre d'anciens combattants de la deuxième guerre mondiale y furent admis en 1946. L'étude fut entreprise pour déterminer si l'incidence accrue de l'infection était due à une exposition excessive à la tuberculose durant le service militaire, ou au fait que ces anciens combattants avaient quelques années de plus que les étudiants ordinaires.

Divers facteurs, y compris l'exposition à la tuberculose, l'infection pulmonaire antérieure, les données radiologiques et le diamètre de la réaction à la tuberculine sont étudiés.

Sur un nombre de 12.415 étudiants testés, 23,3% ont réagi à la tuberculine. Sur ce nombre total, 9.062 étaient des anciens combattants dont 27% avaient des réactions positives; 3.353 n'étaient pas des anciens combattants et 13,4% d'entre eux avaient des réactions positives. A quelques exceptions près, les différences d'âge des anciens combattants et des non combattants étaient suffisantes pour expliquer l'incidence plus élevée de sujets à réaction positive parmi les anciens combattants.

Aucune région particulière du service à l'étranger n'était impliquée dans l'élévation significative du taux des sujets réagissant à la tuberculine. Durant la guerre, un grand nombre d'anciens combattants résidaient dans certaines régions du monde où la tuberculose régnait parmi les civils. Il est évident que ceux qui avaient la responsabilité du personnel des services armés ont fait un excellent travail le protégeant contre le bacille tuberculeux.

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REFERENCES

(1) Lees, H. D., and Myers, J. A.: Tuberculous infection among adults, Am. Rev. Tuberc., 1930, *21*, 532.

(2) Myers, J. A., Boynton, R. E., and Diehl, H. S.: Tuberculosis among university students: A thirty-five year experience, Ann. Int. Med. (in press).

(3) Myers, J. A., Boynton, R. E., Kernan, P., Cowan, D., and Jablon, S.: Sensitivity to fungal antigens among students at the University of Minnesota, Am. Rev. Tuberc., 1956, *73*, 620.

(4) Linder, F. E., and Grove, R. D.: Vital Statistics Rates in the United States, 1900–1940, United States Government Printing Office, 1947, pp. 69–71

(5) Period Reports of Physical Examination, Vol. 3: Summary and Detailed Reports,

November, 1940, September, 1945, National Headquarters, Selective Service System, undated.

(6) COHEN, B. M., AND COOPER, M. Z.: A Follow-up Study of World War II Prisoners of War, U. S. Government Printing Office, September, 1954.

(7) LONG, E. R., AND JABLON, S.: Tuberculosis in the Army of the United States in World War II, U. S. Government Printing Office, May, 1955.

(8) PALMER, C. E., AND PETERSON, O. S.: V. Doubtful reactions to tuberculin and to histoplasmin, Pub. Health Rep., 1950, *65*, 1.

(9) PALMER, C. E., FEREBEE, S. H., AND PETERSON, O. S.: VI. Geographic differences in sensitivity to tuberculin as evidence of nonspecific allergy, Pub. Health Rep., 1950, *65*, 1111.

(10) TAYLOR, W. F.: Mathematic statistical studies on the tuberculin and histoplasmin skin tests, Human Biol., 1951, *23*, 1.

TUBERCULIN SENSITIVITY IN THE AGED[1]

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INTRODUCTION

A recent study of tuberculin testing, conducted by the Department of Health of the City of New York (test dose 0.1 mg. of Old Tuberculin (OT)) and reported by Robins and associates (1), revealed that sensitivity in residents of homes for the aged decreased about 5 per cent with each decade after the age of sixty-five, reaching a reactor percentage of approximately 50 per cent at eighty-five and older. Heaf (2) has recorded numerous studies in many parts of the world which reveal high percentages of negativity in the aged which tend to increase with advancing years.

The purpose of this study is to determine the significance of this waning tuberculin sensitivity in the aged. Under consideration are the views: that a change in the local skin circulation or capillary permeability might be responsible for the changed reaction; or that there is a true and complete loss of sensitivity; or that a low level of sensitivity exists, not responsive to the usual testing methods, which might be detected and/or stimulated by the intradermal inoculation of BCG vaccine.

The question of low-level or waning tuberculin sensitivity in animals and the effect of reinfection upon cutaneous hypersensitivity were studied by Willis (3) and Krause (4). They found that tuberculin sensitivity in guinea pigs, created by inoculation of R1 (an organism that could not produce progressive disease), which had waned considerably two years after infection, could be stimulated by reinfection with virulent organisms (H37). Reinfection was achieved by subcutaneous as well as by intracutaneous inoculation. In humans, Canetti and Lacaze (5) showed that thirteen of thirty subjects more than fifty years of age, negative to a 1:100 dilution of tuberculin, developed positive reactions upon retesting six days after subcutaneous inoculation of BCG vaccine. Local reactions were still small thirty days after BCG inoculation, and large local Koch reactions were rarely observed. Troisier and co-workers (6) studied aged subjects, some of whom were tuberculin negative and some of whom had doubtful reactions to tuberculin in a dilution of 1:10. BCG vaccine was inoculated by both intradermal and scarification methods. Three of sixteen negative reactors, and four of ten doubtful reactors became positive seven days after BCG inoculation. Under these conditions, the incidence of the resensitization phenomenon was not impressive. Whether or not the lack of sensitivity was the result of a deficiency of antigenic stimulation and whether or not BCG vaccine could provide that stimulation appear to be problems peculiarly acute in aged persons.

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Plan of Investigation

Thirty-six persons of Norwegian and Swedish origin in two homes for the aged, and 6 tuberculous patients receiving multiple-drug chemotherapy (at least two major antituberculous drugs), who would provide certain allergy as evidenced by positive Mantoux reactions and accelerated Koch phenomena, were studied.

All persons were skin tested throughout the study with 0.1 mg. of OT prepared by the New York City Department of Health Laboratories. The left forearm was used throughout except when noted. The BCG vaccine was an intradermal preparation shipped weekly to the Department of Health by the Henry Phipps Institute of Philadelphia. Lot No. 457 was used for all persons, and 0.1 ml. was injected intradermally in the deltoid area of the left arm.

The tuberculous patients were Mantoux tested originally by the chief resident of the tuberculosis service of the Willard Parker Hospital. The tests were read at forty-eight hours. BCG vaccine was given to all immediately after the reading. All of the aged were given Mantoux tests and received BCG vaccine immediately after the reading at seventy-two hours. All of the negative reactors and several positive reactors chosen at random were inoculated.

Studies were made as follows: Vaccination sites were examined at four, fourteen, and twenty-eight days after BCG inoculation, while the postinoculation Mantoux tests were performed at eleven and twenty-five days when indicated, and read at fourteen and twenty-eight days, respectively. A final vaccination site reading was made two months after BCG inoculation in the aged, and one month after inoculation in the tuberculous group. A positive reaction to a tuberculin test was considered to be an area of induration of 5 mm. or more in diameter at forty-eight or seventy-two hours. All but one of the aged and all of the tuberculous patients had chest roentgenograms.

Observations

Twenty-six white females and 10 white males, between seventy-three and ninety-four years of age, averaging eighty-three years of age, received Mantoux tests which were read at seventy-two hours, except for one test which was read at one week. Eleven of the reactions were positive, and 25, including the one read at one week, were negative. Twenty-four negative reactors received BCG. Ten positive reactors, whose reaction averaged 11 mm., composed of 5 aged subjects and 5 male tuberculous patients (3 white and 2 Chinese), the latter 5 between forty-two and seventy-one years of age (averaging fifty-nine years of age), received 0.1 ml. of the same lot of vaccine.

In tables 1, 2, and 3, the tuberculous reactors are grouped with the aged positive reactors who received BCG to form one original Mantoux positive group. These tables contain the results of the Mantoux responses at four, fourteen, and twenty-eight days after BCG inoculation and their related reactions at the vaccination sites.

The evidence (table 1) for the resensitizing effects of BCG is apparent as early as the four-day examination. Three Mantoux positive sites, averaging 12 mm., appeared where 24 negative reactions had previously existed. Moreover, there was a sharp division of the vaccination sites into two groups, one group of 9 (mean size 16 mm., and all of which were 10 mm. or more), showing a large, erythematous accelerated type resembling that seen following the vaccination of the 10 positive reactors, and another group of 15 (mean size 5 mm., and all

TABLE 1

MANTOUX RESPONSES AND RELATED VACCINATION SITE RESPONSES, AT FOUR DAYS* AFTER BCG INOCULATION

Original Mantoux Group	Mantoux Responses				Vaccination Site Responses							
									Ulcer or Crust			
									Yes		No	
	Number	Number Negative	Number Positive	Mean Size Positive	10 Mm. or More	Mean Size	Less than 10 Mm.	Mean Size	10 Mm. or More	Less than 10 Mm.	10 Mm. or More	Less than 10 Mm.
Negative	24	21	3	12	9	16	15	5	0	0	9	15
Positive	10	1	9	11	10	20	0	—	1	—	9	—

* Re-examination only of old Mantoux site.

TABLE 2

MANTOUX RESPONSES AND RELATED VACCINATION SITE RESPONSES, AT FOURTEEN DAYS* AFTER BCG INOCULATION

Original Mantoux Group	Mantoux Responses				Vaccination Site Responses							
									Ulcer or Crust			
									Yes		No	
	Number	Number Negative	Number Positive	Mean Size Positive	10 Mm. or More	Mean Size	Less than 10 Mm.	Mean Size	10 Mm. or More	Less than 10 Mm.	10 Mm. or More	Less than 10 Mm.
Negative	22	10					10	6	—	0	—	10
			7	13	7	16	—	—	7	—	0	—
			5	9	—	—	5	6	—	2	—	3
Positive	10	—	—	—	10	15	0	—	9	—	1	—

* Mantoux tests of positive reactors not repeated.

less than 10 mm.) showing a small, nonaccelerated purplish-hued macular or papular type.

Further evidence (table 2) for the resensitization phenomenon was brought out at the fourteen-day examination when 12 of 22 original Mantoux negative reactors were found positive on retesting. Of the 12 positive reactions, 7, averaging 13 mm., developed in subjects with large accelerated vaccination sites of 10 mm. or more (mean size 16 mm.). All of the reactions had ulcerated at this time, and bore a striking resemblance to the vaccination sites of the 10 positive reactors (mean size 15 mm.), the sites of 9 of which had ulcerated. The remaining 5 (in subjects in whom resensitization is open to question), averaging 9 mm., were derived from small vaccination sites of less than 10 mm. (mean size 6 mm.), only 2 of which had ulcerated. These resembled the sites of the 10 original Mantoux negative reactors who failed to convert at this time. The latter group of 10 was derived from sites all of which were less than 10 mm. (mean size 6 mm.), none of which had ulcerated.

TABLE 3

MANTOUX RESPONSES AND RELATED VACCINATION SITE RESPONSES, AT TWENTY-EIGHT DAYS* AFTER BCG INOCULATION

Original Mantoux Group	Mantoux Responses				Vaccination Site Responses							
									Ulcer or Crust			
									Yes		No	
	Number	Number Negative	Number Positive	Mean Size Positive	10 Mm. or More	Mean Size	Less than 10 Mm.	Mean Size	10 Mm. or More	Less than 10 Mm.	10 Mm. or More	Less than 10 Mm.
Negative	10	1	9	10	4	11	6†	6	2	2	2	2

* Mantoux test on right forearm.

† Two vaccination sites not described.

The 10 subjects of the original Mantoux negative group who had failed to convert at the fourteen-day retesting were again tested at twenty-eight days. The results, together with related vaccination site responses, may be seen in table 3.

At twenty-eight days, true BCG conversion had taken place in the aged as evidenced by positive Mantoux reactions and an increase in size (mean of 8 mm.), with or without ulceration, of the local vaccination site. It would seem that the state of vascularity of the skin and deeper tissues in the aged does not prevent normal response to antigenic stimuli.

At two months after BCG inoculation, final examination was made of the vaccination sites of the aged subjects. Of 22 original Mantoux negative reactors, 19 had vaccination sites which had healed completely, while 3 had sites which were draining. Six of the 19, averaging 7 mm., had accelerated; 9, averaging 6 mm., had not accelerated; while 4, averaging 5 mm., were of doubtful acceleration. The 3 draining sites, the first of 10 mm., the second of 10 mm., and the third of 5 mm., were found in the accelerated, the nonaccelerated and the doubtful groups, respectively. Of 5 original Mantoux positive reactors, 4 had sites, averaging 10 mm., which had healed completely, while one site of 6 mm. continued to drain.

Three tuberculous patients had their final examinations at one month after BCG inoculation. Two sites, one of 8 mm. and the other of 7 mm., had healed completely, while one of 10 mm. continued to drain. In general, the largest vaccination sites throughout the period of the experiment continued to be those of the accelerated reactors, particularly those of the tuberculous patients.

Thirty-five of the 36 aged subjects had chest roentgenograms. All were negative for pulmonary disease.

DISCUSSION

The results lend support to the view that allergy is not lost in the aged, at least to the extent observed in routine testing methods. The results follow the pattern of the early animal experiments of Krause and Willis, and later experi-

ments of Canetti and Lacaze, and Troisier, Sifferlen, and MacLouf in aged humans.

In the animal experiments of Krause and Willis, virulent organisms were used to resensitize animals initially infected with relatively avirulent organisms, while the studies in aged humans were carried out with avirulent bovine organisms as the resensitizing agents. Canetti and Lacaze have also demonstrated that tuberculin can resensitize, although not to the same degree as BCG vaccine.

In the later studies in aged humans by Canetti and Lacaze, and Troisier and associates, the evidence for the resensitization phenomenon revolves around the response to the tuberculin skin test, while in the present studies the local response to the sensitizing agent as well as to tuberculin provided the evidence. Although late responses to tuberculin are known, a positive Mantoux test which develops without retesting four days after BCG inoculation at the site of an originally negative response is strong evidence of previous infection. This occurred in 3 of the present cases, as has been noted. (Similar observations have been made following mass inoculation of children of school age (7).) Following retesting, however, several problems develop. If Mantoux retesting within four or five days after BCG inoculation results in a positive response, it may be safely assumed that resensitization has taken place. At fourteen days, however, a positive Mantoux test is difficult to interpret. Positive responses have been reported as early as eight days after inoculation, and the present writers have not infrequently observed positive reactions at fourteen days.

Under such conditions, the key to the phenomenon of resensitization is the local reaction to BCG. Within a few days after the stimulating agent is introduced, the local area in the previously infected subject becomes large, red, and indurated. It reaches its largest size within two weeks, is generally ulcerated at that time, and then slowly recedes. This was found in the original positive reactors, including the tuberculous patients, and in 7 of the original negative reactors in the aged.

The 5 aged subjects in whom resensitization was considered doubtful had indeed developed a positive Mantoux response at fourteen days, but in the absence of a large, ulcerated vaccination site, could not truly be considered to have been previously infected.

The site of BCG inoculation in a previously noninfected subject grows slowly and reaches its greatest size in about a month, although never reaching the size of the accelerated site, and then recedes slowly for another month. It may or may not ulcerate, and does not produce as intense an erythema as the resensitized site.

Thus 7 of 22 aged negative reactors were shown to have a low level of sensitivity which could be stimulated by intradermal inoculation of BCG vaccine. It is probable that the low level of sensitivity which can be detected in this way occurs in increasing incidence with advancing age. The aged studied, however, may be unusual in the sense that they had lived a sheltered existence for many years. Lack of contact with tubercle bacilli for long periods may have resulted in the loss of the elevating or booster effect of living or dead organisms or perhaps fractions

thereof. The increasing loss of sensitivity with each decade after sixty-five, which was reported in the Robins study, referred only to residents of homes for the aged (56.4 per cent positive at sixty-five and older), while the same study revealed a much higher incidence of positivity in chest clinic patients of sixty-five and older (69.1 per cent).

The negative reactors who did not have accelerated reactions and who apparently had never been infected could have allergy elicited under normal conditions by the usual dosage of BCG vaccine. This last group of approximately the same age as the resensitized and the doubtful groups may have lost its allergy completely. It is suggested that roentgenographic studies of calcium deposits and the traditional pathologic and bacteriologic studies in addition to resensitization experiments may throw light on the question of whether allergy can be completely lost.

In his recent review, J. Pepys (8) has shown how alteration of local vascular responses can either inhibit or prolong persistence of the tuberculin reaction: "The local persistence of tuberculin is influenced by lymphatic absorption. When absorption is decreased, reactions are enhanced and vice versa." There is no reason to believe, a priori, that conditions in the aged favor increased lymphatic absorption, although decreased availability of cells which are responsible for the production of allergy would seem to be a possible factor. Under the conditions of this experiment, however, it would seem that a positive Mantoux test in association with an accelerated local reaction within fourteen days after BCG inoculation in a previously negative reactor is more likely to be related to the elevation of a low allergy level than to any alteration of local vascular activity.

Summary

Following BCG inoculation of 22 relatively isolated aged subjects, who were negative to tuberculin when routine testing methods were employed, 7 were resensitized. This was evidenced by a positive Mantoux test plus an accelerated and accentuated vaccination site reaction within two weeks after BCG inoculation.

A positive Mantoux test read fourteen days after BCG inoculation, in the presence of a small local reaction, is of doubtful significance in terms of the resensitization phenomenon.

Aged negative tuberculin reactors, who apparently had never been infected, had allergy elicited under normal conditions by the usual amounts of BCG vaccine.

More delicate methods of testing sensitivity than the routine ones in general use are necessary before complete negativity to tuberculin can be considered to have been established. Changes in skin vascularity and capillary permeability apparently play no part in this phenomenon.

These studies make it likely that the low level or absence of tuberculin allergy commonly observed in the aged is a manifestation of deficiency of antigenic stimulus rather than of abnormal immunologic and physiologic responsiveness.

Sumario

Sensibilidad a la Tuberculina en los Ancianos

Después de inocular con BCG a 22 sujetos de edad avanzada y relativamente aislados, que se habían mostrado negativos a la tuberculina con los métodos corrientes de comprobación, 7 se resensibilizaron. Puso esto de manifiesto una Mantoux positiva y además una reacción acelerada y acentuada en el sitio de la vacunación en término de dos semanas de la inoculación de BCG.

Una Mantoux positiva leída a los catorce días de la inoculación de BCG, en presencia de una pequeña reacción local, reviste importancia dudosa en términos del fenómeno de resensibilización.

En ancianos negativos, que aparentemente nunca se habían infectado, provocaron alergia en condiciones normales las cantidades habituales de vacuna BCG.

Antes de poder considerar la negatividad indudable, se necesitan métodos más delicados que los de empleo general corriente para comprobar la sensibilidad. Las alteraciones de la vascularidad cutánea y de la permeabilidad capilar no desempeñan al parecer papel alguno en este fenómeno.

Estos estudios acentúan la probabilidad de que el bajo nivel o la falta de alergia tuberculínica observados comúnmente en los ancianos sean una manifestación de deficiencia de estímulo antigénico más bien que de anómala sensibilidad inmunológica y fisiológica.

Resume

Sensibilité à la tuberculine chez le vieillard

Après inoculation de BCG à 22 sujets âgés, relativement isolés et présentant des tests négatifs à la tuberculine effectués par les méthodes classiques, 7 d'entre eux furent resensibilisés. Ceci fut démontré deux semaines plus tard par un test de Mantoux positif, plus une réaction accélérée et accentuée au siège de la vaccination par le BCG.

En présence d'une réaction locale légère, le test de Mantou positif observé quatorze jours après l'inoculation du BCG n'a qu'une valeur douteuse à l'égard du phénomène de la resensibilisation.

L'allergie a été provoquée dans des conditions normales par les doses habituelles de vaccin BCG chez des sujets âgés anergiques, qui paraissaient n'avoir jamais été infectés.

Des méthodes plus délicates que les méthodes classiques utilisées pour les tests de sensibilité doivent être pratiquées avant qu'on puisse considérer comme certaine la négativité complète du test. Des modifications de la vascularisation cutanée et la perméabilité capillaire ne paraissent pas jouer un rôle dans ce phénomène.

Ces recherches permettent de penser que le faible degré ou l'absence de l'allergie à la tuberculine, habituellement observé chez le vieillard, est une manifestation de déficience du stimulus antigénique plutôt qu'une réaction immunologique et physiologique anormale.

Acknowledgment

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REFERENCES

(1) Robins, A. B., Abeles, H., Aronsohn, M. H., Glass, R., Goldberg, S. I., Konterwitz, H., Levine, I., and Schwartz, S.: Tuberculin-testing studies in New York City, Am. Rev. Tubere., 1954, *69*, 1057.

(2) Heaf, F. R. G.: Tuberculosis in geriatrics, J. Roy. Inst. Pub. Health, 1955, *18*, 324.
(3) Willis, H. S.: Studies on immunity to tuberculosis: The waning of cutaneous hypersensitiveness to tuberculin and the relation of tuberculoimmunity to tuberculoallergy, Am. Rev. Tubere., 1928, *17*, 240.
(4) Krause, A. K.: Experimental studies on the cutaneous reaction to tuberculoprotein: I. Factors governing the reaction, J. med. Res., 1916, *35*, 1.
(5) Canetti, G., and Lacaze, H.: Données Nouvelles sur L'Evolution et la Signification de L'Allergie Tuberculinique. Les Grandes Tendances Evolutives de L'Allergique Tuberculinique chez les Sujets non Tuberculeux. Allergie et Reinfection, Ann. Inst. Pasteur, 1940, *65*, 435.
(6) Troisier, M. J., Sifferlen, and MacLouf, M. A. C.: Anergie Tuberculinique Senile et Phenomene de Baldwin-Gardner-Willis, Presse méd., 1942, *50*, 609,
(7) Ustvedt, H. J.: Local reaction in BCG vaccination, Bull. WHO, 1950, *2*, 441.
(8) Pepys, J.: The relationship of nonspecific and specific factors in the tuberculin reaction: A review, Am. Rev. Tubere., 1955, *71*, 49.

PERICARDIAL BIOPSY[1, 2]

Role in Diagnosis and Treatment of Chronic Pericarditis

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INTRODUCTION

Chronic pericarditis is an unusual condition, but it occurs more commonly than is generally believed. By tradition, pericarditis of undetermined etiology is suspected of being tuberculous in nature. Since available diagnostic procedures are extremely limited in this disease, a presumptive diagnosis alone often has been the basis for treatment. In bacterial pericarditis the findings on examination of the pericardial fluid may be diagnostic, but in tuberculous pericarditis the findings on direct smears, cultures, and guinea pig inoculations of the specimens of fluid often are negative. Histopathologic and bacteriologic examinations of the diseased pericardium itself may be of considerable assistance in diagnosis and should be considered in selected cases. An operative technique for pericardial biopsy and for treatment of pericardial effusion is presented, and results in 22 cases are reported.

TECHNIQUE

The method as employed by the writers has two primary objectives: (*1*) to obtain pericardial tissue for histopathologic and bacteriologic studies, and (*2*) to create a pleural pericardial hiatus, or "window," to establish drainage of effusion into the adjacent pleural space. Both objectives can be accomplished by excision of a small pericardial disc in the dependent portion of the pericardial sac. The technique itself is simple, and may be employed even in patients who are gravely ill.

Pericardial biopsy is a simple surgical procedure that can be performed in a standard operating room and requires only a few special instruments. The anesthesia required is minimal, but it is of extreme importance because many of these patients are seriously ill. Since the diagnosis of chronic pericarditis is usually established in hospitalized patients, the procedure of pericardial biopsy should not add appreciably to the over-all hospital stay.

Anesthesia: Pentobarbital sodium is given intravenously immediately prior to induction; atropine should be avoided, as it produces tachycardia. After the barbiturate has been administered, topical cocaine (4 per cent) is applied to the pharynx, vocal cords, and trachea. With adequate cocainization an endotracheal tube can be inserted and oxygen administered under mild positive pressure. It may be advisable to use thiopental sodium as a supplement, but the lightest possible anesthetic plane must be maintained. After prepara-

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[2] Presented before the Medical Session, as part of *Section 6B*, at the annual meeting of the National Tuberculosis Association, New York, New York, May 23, 1956.

tion of the anterior thorax, an intercostal block is performed over the selected site of incision. Deep anesthesia is unnecessary and inadvisable.

Operation: The incision is usually made in the left anterior thorax using the fourth or fifth interspace (figure 1*a* and *b*). The left pleural space is entered through an intercostal incision; a baby rib spreader will provide adequate exposure. The underlying lung is fully expanded by positive pressure oxygen at all times. With exposure of the pericardial sac, the

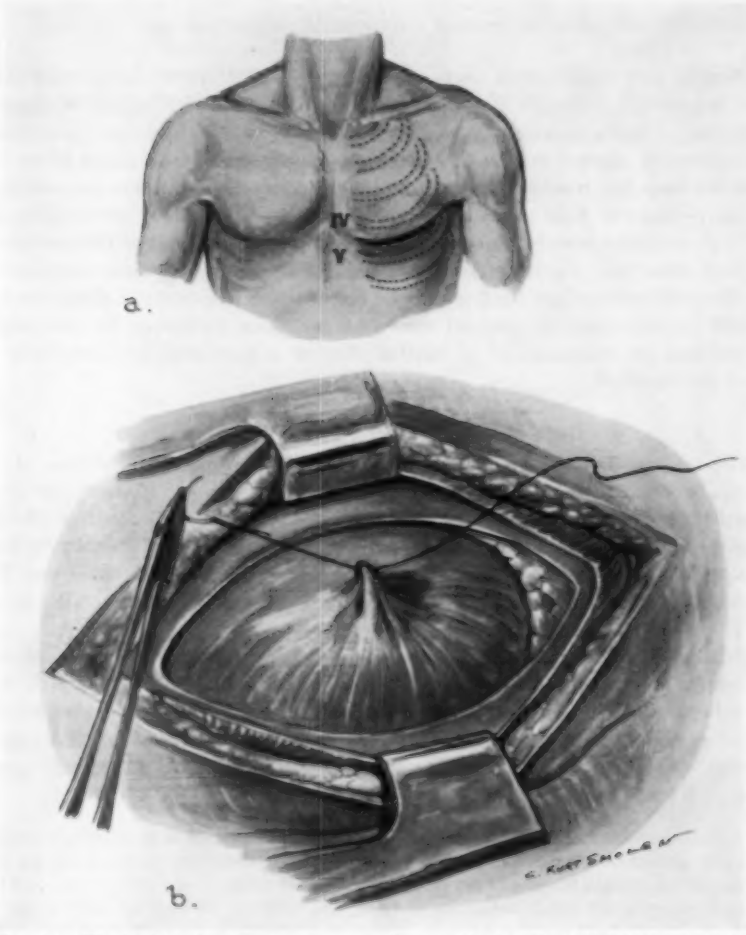

FIG. 1. *a*. The incision is submammary, usually over the fourth or fifth interspace of the left anterior chest wall. Occasionally it will be advisable to employ a right thoracotomy instead; the approach is identical to its counterpart on the left. *b*. The transpleural approach to the pericardium affords a large exposure through a relatively small thoracotomy incision. The pericardial fat is easily dissected away from the pericardium after selection of the biopsy site. A single traction suture is employed to elevate the pericardium.

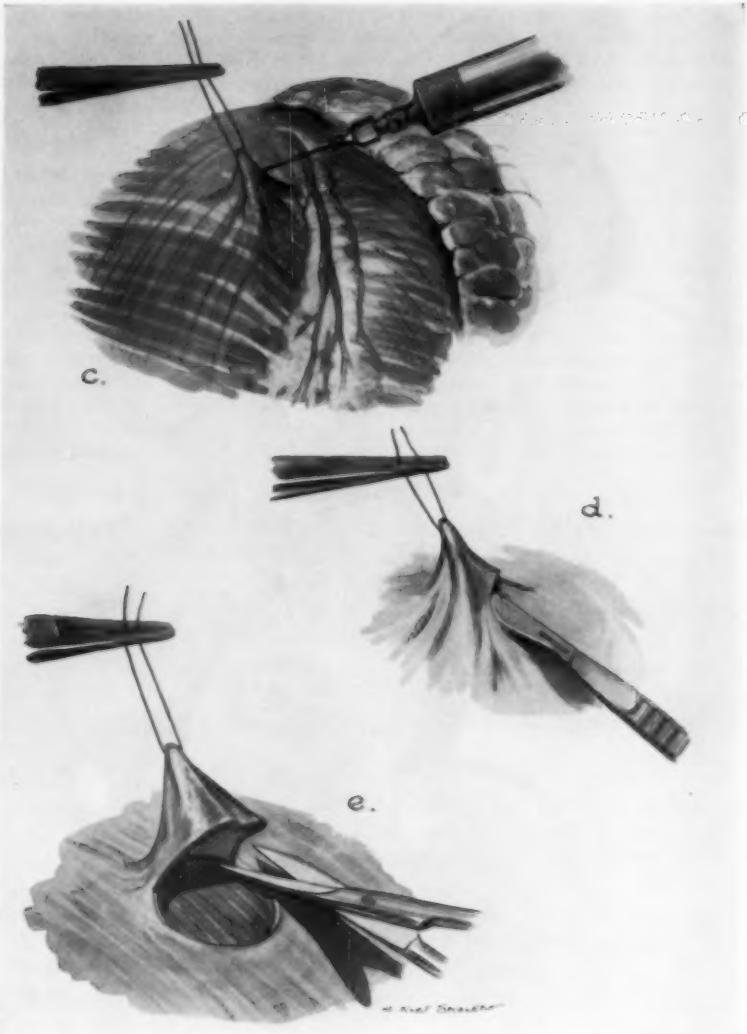

FIG. 1. *c*. If fluid is present, a specimen may be obtained under sterile conditions for separate bacteriologic and cytologic studies. When fluid sufficient to produce tamponade is present, abrupt decompression is performed. *d*. The pericardial biopsy is outlined by the knife; the size ranges from a disc comparable to a 25-cent piece to that comparable to a silver dollar. *e*. If there is adherence between pericardium and epicardium, the specimen may be decorticated by scissor dissection. After removal, the specimen is divided under sterile conditions; one part is used for histopathologic and the other part for bacteriologic studies.

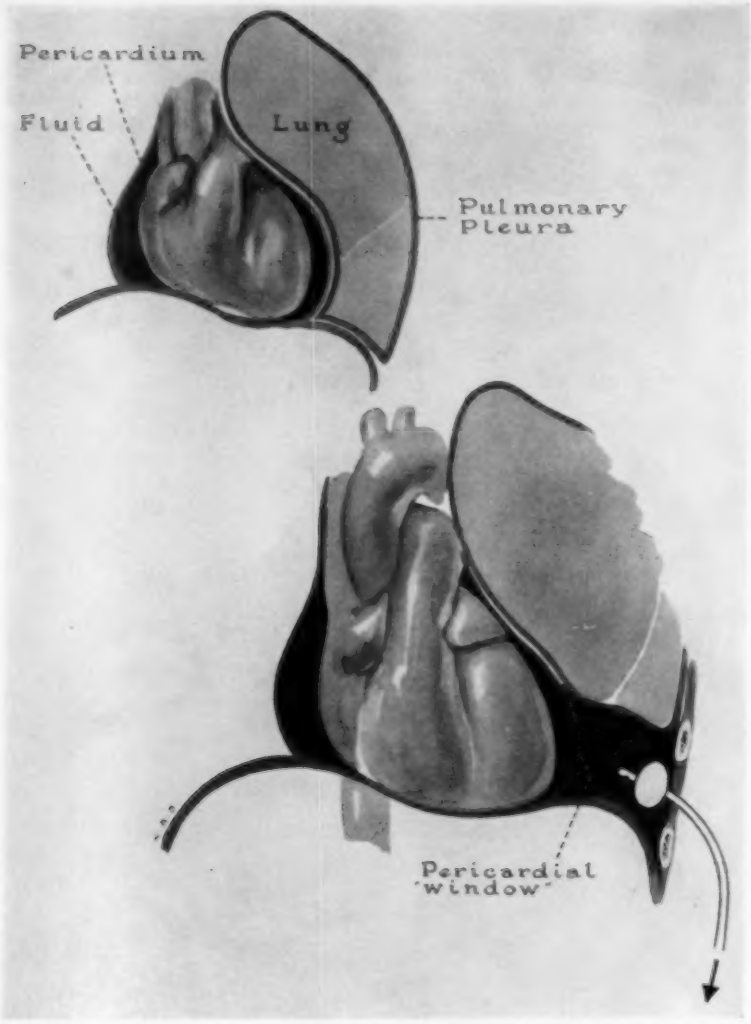

FIG. 2. Schematic representation of the pericardium containing effusion, and its relation to the left pleural space. Removal of the pericardial biopsy specimen at the apex of the pericardial sac creates open, dependent drainage into the adjacent pleural space; the latter is drained by an intercostal catheter connected to waterseal suction.

Use of this drainage technique has permitted relief of tamponade and prompt reduction in size of the pericardial silhouette. In no case has there been evidence to suggest transmission of pericardial disease to the previously uninvolved pleural space.

site of biopsy is selected near the apex of the heart and anterior to the phrenic bundle. The overlying pericardial fat may be removed for added exposure and a mattress suture then placed through the pericardium at the selected site and used for traction elevation. If fluid is present in the pericardial space, specimens may be taken by needle aspiration for bacteriologic and cytologic studies (figure 1*c*). The pericardial specimen for biopsy is then outlined by a knife blade and excised with surgical scissors (figure 1*d* and *e*). If the pericardium is adherent to the underlying epicardium, scissor dissection is employed to decorticate the specimen. With sterile precautions the specimen is divided; one part is used for histopathologic examination, and the other is placed in a sterile tube for bacteriologic studies. No effort is made to close the circular pericardotomy.

If pericardial effusion is present, it may be completely aspirated with removal of the biopsy specimen (figure 2). No difficulty has been encountered with sudden release of large amounts of tamponade fluid. Since the biopsy specimen is taken from a dependent site, further accumulation of fluid may easily drain into the adjacent left pleural space. This fluid in turn may escape by a catheter drainage tube brought out through a stab wound in the lateral or anterior chest wall and connected to a waterseal suction apparatus.

Postoperative care: Since the thoracotomy incision is small and anterior, there is usually little significant discomfort in the postoperative phase. The catheter drain is removed by the fifth or sixth day unless drainage persists. Selection of antimicrobials or specific medications for treatment of the underlying pericarditis depends upon the individual case.

Review of Cases

During the past six years, 22 patients have undergone pericardial biopsy at the Cleveland Clinic (1). There were no operative fatalities or hospital deaths in this series, although several of the patients died later of their disease.

In 2 cases, culture of the pericardial tissue yielded tubercle bacilli; it is of particular interest that the microscopic appearance of the tissue was not characteristic and was reported as showing chronic inflammatory changes only (2). In another case, culture of the pericardial fluid was sterile, but on culture of the tissue a pure strain of *Streptococcus viridans* was obtained; the patient recovered after prolonged penicillin therapy.

There were 3 cases of pericardial effusion due to unusual causes. One patient had a massive bloody effusion that resulted from intrapericardial metastases of a previously resected thymic carcinoma; this patient responded well to mechanical drainage and radiation therapy to the mediastinum, and is apparently well three and a half years after treatment. Another patient developed an unusual pericardial effusion approximately one year after radiation therapy for a resected thymic tumor; the tissue showed microscopic evidence of radiation fibrosis and there has been no recurrence of the effusion since surgical drainage. The third patient demonstrated a true chylopericardium without pleural effusion. This case has been reported previously (3).

There were 8 cases of chronic hemorrhagic pericarditis in which culture of the fluid and pericardial tissue was sterile; and the histopathologic examination showed only nonspecific inflammation. This hemorrhagic pericarditis of undetermined etiology is of particular interest, because the residual blood elements produce great thickening of the pericardium and deposit a peculiar shaggy exudate over the entire surface of the heart and pericardial sac. It is possible, although unproved, that this disease initially is provoked by an acute viral infection. It

is also of interest that the unknown inflammatory disease may provoke severe hemorrhagic changes within the pericardium without exciting any response in the adjacent pleurae. In each of these cases containing residual blood elements and thickening of both pericardium and epicardium, there was a significant amount of serous fluid present under sufficient pressure to produce some mild degree of tamponade with slight elevation of the venous pressure. It is believed that this fluid collects as a result of osmotic attraction; in other words, the serous fluid is drawn into the area of recent hemorrhage in a manner similar to that which occurs in subdural hematoma or chronic hemothorax. This theory raises the interesting possibility that severe hemorrhagic pericarditis may be the precursor of constrictive calcific pericarditis when the residual blood elements are not absorbed.

Ten patients were treated with antimicrobial therapy, usually streptomycin and PAS, although only 2 of these patients had a positive bacteriologic culture. The therapy was given on an empiric basis and apparently contributed little to the ultimate recovery of those in whom the cultures were sterile.

Discussion

There is an appreciable risk in paracentesis for the diagnosis of pericardial effusion. Cardiac arrhythmia, syncope, and laceration of the myocardium and coronary vessels have been reported as complications. Paracentesis in itself has definite limitations, because it is essentially a "blind" procedure. Failure to obtain fluid does not prove it to be absent, nor does a sterile culture of a pericardial fluid necessarily exclude the presence of a specific organism within the pericardium. Although paracentesis in itself may be a valuable procedure in certain cases, it can never approach pericardial biopsy in value.

The creation of a pleural pericardial window obviates repeated paracenteses, in addition to providing material for laboratory study. The objection is constantly raised that a disease confined to the pericardial space will be transmitted to the adjacent pleural space by this form of drainage. However, this has not been proved. Careful observation of all patients who have undergone this type of surgery during the past six years has shown no instance where aggravation or dissemination of the disease has occurred. Although most of these patients have had empiric antimicrobial treatment after surgery, it seems most likely that local tissue resistance has been the major factor in preventing extension of pericardial disease to the adjacent pleural space. It has been demonstrated by others that the pleura has a more absorptive surface than does the pericardium (4, 5).

Summary

A technique of pericardial biopsy is described. This operation has been performed in a series of 22 cases at the Cleveland Clinic. Pericardial biopsy permits direct visualization within the pericardial sac; histopathologic examination and cultures of the diseased pericardium and fluid may establish the etiology of chronic pericarditis.

Creation of a pleural pericardial hiatus offers a rational therapy for chronic pericarditis associated with effusion.

Sumario

La Biopsia Pericardíaca: Papel en el Diagnostico y Tratamiento de la Pericarditis Cronica

Descríbese una técnica para la biopsia pericardíaca, operación esta ejecutada en una serie de 22 casos en la Clínica de Cléveland. La biopsia pericardíaca permite la visualización directa dentro del saco pericardíaco; el examen histopatológico y los cultivos del pericardio afectado y del líquido quizás establezcan la etiología de una pericarditis crónica.

La creación de una abertura pericardíaca pleural ofrece una terapéutica raciona! para la pericarditis crónica asociada con derrame.

Resume

Biopsie péricardique: Son rôle dans le diagnostic et le traitement de la péricardite chronique

Description d'une technique de biopsie péricardique. L'opération a été pratiquée dans une série de 22 cas dans la Clinique de Cleveland (Etats-Unis). La biopsie péricardique permet la visualisation directe à l'intérieur du sac péricardique; l'examen anatomo-pathologique et les cultures des tissus et du liquide du péricarde affecté peuvent permettre d'établir l'étiologie de la péricardite chronique.

La création d'une communication pleuro-péricardique constitue un traitement rationnel de la péricardite chronique avec épanchement.

REFERENCES

(1) Proudfit, W. L., and Effler, D. B.: Diagnosis and treatment of cardiac pericarditis by pericardial biopsy, J. A. M. A., 1956, *161*, 188.

(2) Janovsky, R. C., Boettner, J. F., Van Ordstrand, H. S., and Effler, D. B.: Recurrent tuberculous pericarditis, Ann. Int. Med., 1952, *37*, 1268.

(3) Groves, L. E., and Effler, D. B.: Medical intelligence: Primary chylopericardium, New England J. Med., 1954, *250*, 520.

(4) Drinker, C. K., and Field, M. E.: Absorption from pericardial cavity, J. Exper. Med., 1951, *53*, 143.

(5) Stewart, H. J., Crane, N. F., and Deitrick, J. E.: Absorption from pericardial cavity in man, Am. Heart J., 1938, *16*, 198.

(6) Williams, C., and Soutter, L.: Pericardial tamponade: Diagnosis and treatment, A. M. A. Arch. Int. Med., 1954, *94*, 571.

STUDIES ON ISONIAZID-RESISTANT STRAINS OF MYCOBACTERIUM TUBERCULOSIS[1]

III. The Effect of Peroxide in Culture Media

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Introduction

Several workers (1–5) have shown that strains of *Mycobacterium tuberculosis* highly resistant to isoniazid require various additives for growth in certain synthetic media. Middlebrook has shown that these strains frequently lack catalase (6), and he and his co-workers suggested that failure of the strains to grow in these media is due to the presence of peroxide. Support for this came from Bönicke (7), Knox (4), and from this laboratory (8), but results were irregular and did not always appear to be consistent with the above suggestion. For example, Middlebrook's group (3) found that erratic results were obtained unless manganese was added to the media, and in the laboratory of the present writers it was noted (8) that the requirements varied apparently unpredictably so that no all-embracing theory seemed possible.

In the present study the levels of peroxide (believed to be hydrogen peroxide) present in some Proskauer and Beck types of medium have been measured by chemical means, and the roles of the various medium constituents in the formation of the peroxide have been ascertained. The stability of peroxide in media and the activity of various agents as destroyers of peroxide and as promoters of growth of the isoniazid-resistant strains have also been investigated. The minimal inhibitory concentrations of hydrogen peroxide for the strains under investigation have been determined, and the bactericidal effect of hydrogen peroxide on one of them has been studied.

Materials and Methods

Media: The media studied were Proskauer and Beck (9), Youmans medium (10), and Fisher's medium (11).

Additions to media: Sterile human serum was used. Bovine albumin, fraction V, was Seitz-filtered as a 10 per cent solution. Cobalt chloride (2 per cent solution), reductone (1 per cent), ascorbic acid (1 per cent), fructose (1 per cent), sodium glutamate (1 per cent), methylene blue (1 per cent), and cysteine hydrochloride (1 per cent) were "sterilized" by bringing to the boiling point. Charcoal and Adams catalyst were dry sterilized. The other solutions were regarded as self-sterile.

The additions were diluted in the medium under investigation, added to this medium to give the required concentration, and kept for the required length of time before inoculation. Charcoal and Adams catalyst were removed by paper filtration under sterile conditions before inoculation of the medium.

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TABLE 1

CONCENTRATIONS OF PEROXIDE IN SOME PROSKAUER AND BECK TYPES OF MEDIUM

Medium	Peroxide
	γ per ml.
Proskauer and Beck, *Batch A:*	
Fresh	9.3
3 weeks old*	8.3
11 weeks old*	4.9
Proskauer and Beck, *Batch B:* Fresh	3.0
Proskauer and Beck, *Batch C:* Fresh	1.0
Youmans: Fresh	1.6
Youmans (containing 0.2 per cent ammonium sulfate in place of asparagine): Fresh	0.3
Fisher's: Fresh	0.6
Fisher's (containing 0.04 per cent ammonium sulfate in place of asparagine): Fresh	0.1

* The Proskauer and Beck medium was stored at room temperature in diffuse light.

Strains of tubercle bacilli: The isoniazid-resistant strains have been described previously (8). They were maintained on Löwenstein medium containing 100 γ of isoniazid per ml.

An inoculum of approximately 0.03 mg. (one drop from a Pasteur pipette) was added to each tube of 5 ml. of liquid medium.

Detection of peroxide: The presence of peroxide was shown by liberation of iodine from potassium iodide in acid solution, formation of yellow "pertitanic acid" with titanic sulfate, luminescence with luminol (12), a benzidine-peroxidase test (13), and decolorization of potassium permanganate.

Determination of peroxide: Titanium sulfate reagent was prepared by heating excess titanium dioxide with sulfuric acid to boiling, cooling, adding an equal volume of water, cooling, and centrifuging to clear. One milliliter of this reagent was added to 3 ml. of the analytic solution, the mixture shaken, and its extinction read at 400 mμ after approximately five minutes. Readings were made also on water plus reagent and on the analytic solution plus 50 per cent sulfuric acid. A standard curve was prepared. This enabled accurate results to be obtained over the range 2–40 γ of hydrogen peroxide per ml., and an estimate to be made down to about 0.2 γ per ml.

RESULTS

Concentrations of Peroxide in Proskauer and Beck Types of Medium[2]

As has already been reported (14), Proskauer and Beck medium contains a peroxide, the presence of which was established by the tests already listed. It was concluded from evidence given later that at least the major portion of this peroxide was hydrogen peroxide.

The concentrations of peroxide in some commonly used Proskauer and Beck types of medium are shown in table 1. It will be seen that the amounts varied considerably. Some batches, e.g., batch *A*, showed unusually high concentrations of peroxide. This was traced to the fact that these batches were prepared from one sample of magnesium citrate which contained an abnormally high percentage

[2] The peroxide concentrations given throughout the paper are based on the assumption that all the peroxide present is hydrogen peroxide.

TABLE 2

CONCENTRATION OF PEROXIDE FORMED ON AUTOCLAVING AT TEN POUNDS FOR TEN MINUTES, CONSTITUENTS OF YOUMANS MEDIUM WITH AND WITHOUT MANGANESE

Addition to Aqueous Solution of Inorganic Constituents (pH 6.95)	Peroxide	
	No Mn^{++}	1 γ per Ml. of Mn^{++}
	γ *per ml.*	γ *per ml.*
Glycerol (2 per cent)	Trace?	Trace?
Asparagine (0.5 per cent)	0.0	0.0
Sodium citrate (0.25 per cent)	0.0	6.0
Glycerol + asparagine (as above)	Trace?	Trace?
Glycerol + sodium citrate (as above)	0.3	6.0
Asparagine + sodium citrate (as above)	0.5	6.0
Asparagine + glycerol + sodium citrate (as above)	0.9	6.0

of manganese. Further, it is obvious from the figures in table 1 that replacement of asparagine by ammonium salts led to a considerable reduction in the amount of peroxide formed. Fisher's medium, which contained less asparagine than the other media, also contained less peroxide.

When the media were sterilized by Seitz filtration instead of by autoclaving, no peroxide was found.

Factors Responsible for the Formation of Peroxide in Proskauer and Beck Types of Medium

When various combinations of the organic constituents of Youmans medium were autoclaved separately, at 10 pounds for ten minutes in an aqueous solution of the inorganic components (pH 6.95) in the presence and absence of manganese, the findings in table 2 were obtained. It may be seen that glycerol, asparagine, and citrate by themselves gave rise, at most, to doubtful traces of peroxide. Adding manganese to the glycerol or asparagine solution caused no increase in peroxide formation. When citrate was autoclaved with manganese, however, a considerable quantity of peroxide was formed. Significant amounts of peroxide were formed when either glycerol or asparagine was autoclaved with citrate, while more was formed when both were autoclaved together with the citrate. This would suggest that traces of manganese were present in the asparagine and glycerol although it was measurable only in the case of the asparagine.

Manganese was capable of catalyzing peroxide formation even when present at very low concentrations. A batch of Youmans medium autoclaved without added manganese contained 1.6 γ of peroxide per ml., but when 1.0, 0.1, and 0.01 γ of manganese per ml. were added before autoclaving, the amounts of peroxide were 6.0, 4.4, and 2.9 γ per ml., respectively.

When sodium citrate solution (0.25 per cent) containing 1.0 γ of manganese per ml. was heated for ten minutes at 100°C. in air, 1 γ of peroxide per ml. was formed. Below 100°C. the rate of formation dropped rapidly until at room temperature no measurable amounts of peroxide were found even after long standing. At 37°C. approximately 0.3 γ of peroxide per ml. per day was formed when

TABLE 3

THE EFFECT OF THE CONSTITUENTS OF YOUMANS MEDIUM ON THE CONCENTRATION OF PEROXIDE REMAINING IN A 20 γ PER ML. SOLUTION (pH 6.95), IN THE PRESENCE AND ABSENCE OF 1 γ Mn^{++} PER ML.

Medium Constituents	Peroxide Remaining (γ/ml.)				
	Autoclaved at 10 Pounds for 10 Minutes	Incubated at 37°C.		Incubated at 20°C.	
		7 days	14 days	7 days	14 days
Inorganic salts	19.5	19.1	19.1	19.6	19.7
Inorganic salts + Mn	13.8	14.0	4.2	19.0	14.2
Salts + glycerol	0.2	0.0	0.0	3.6	0.7
Salts + glycerol + Mn	0.0	0.0	0.0	6.3	1.2
Salts + asparagine	11.9	18.0	13.2	18.9	18.5
Salts + asparagine + Mn	11.4	16.4	12.6	20.1	20.1
Salts + citrate	18.3	17.7	16.7	17.8	15.1
Salts + citrate + Mn	22.6	12.2	14.8	20.5	20.4
Salts + glycerol + asparagine	4.7	18.6	12.0	17.9	14.1
Salts + glycerol + asparagine + Mn	2.2	19.7	17.4	20.5	20.4
Salts + glycerol + citrate	15.2	13.4	2.5	18.5	15.0
Salts + glycerol + citrate + Mn	16.7	16.8	19.3	20.1	19.6
Salts + asparagine + citrate	18.7	20.5	20.7	19.8	19.8
Salts + asparagine + citrate + Mn	24.8	20.9	20.9	20.0	20.0
Full medium	17.2	20.1	20.1	20.0	20.0
Full medium + Mn	22.2	20.2	18.7	19.5	19.1

citrate was incubated with 1.0 γ of manganese per ml., and about 0.5 γ per ml. per day when 10.0 γ of manganese per ml. were present.

The autoclave was customarily allowed to steam for about ten minutes before the valves were closed. From the result quoted above it would appear that only a fraction of the peroxide in the medium could be formed during this steaming. Hence, in spite of the low oxygen tensions which must prevail, the greater part of the peroxide must have been formed during the autoclaving.

As has already been reported (14), the ability to catalyze the formation of peroxide in Proskauer and Beck types of medium seems to be confined to manganese.

Stability of Hydrogen Peroxide in Proskauer and Beck Types of Medium

It was found somewhat surprisingly that the peroxide formed in Proskauer and Beck medium was very stable in this medium (table 1). However, added hydrogen peroxide was also very stable in this type of medium (*see* results for Youmans medium at bottom of table 3). In order to discover what medium constituents affect the persistence of hydrogen peroxide, the experiments summarized in table 3 were carried out.

It may be seen that hydrogen peroxide was quite stable in a solution of the inorganic constituents alone (pH 6.95). Asparagine or citrate affected this stability only slightly, except in the autoclave when asparagine increased the destruction considerably. Glycerol removed the peroxide relatively rapidly, but

either asparagine or citrate prevented this removal to a considerable extent. This last effect was rather variable, however, suggesting that some other unknown factor was also involved. When citrate and asparagine were both present with the glycerol, almost complete stabilization of the peroxide was achieved. The effect of manganese was complex. In most combinations it reduced the stability of the peroxide, but in some it increased it. As would be expected, when manganese and citrate were present together, the formation of fresh peroxide confused the picture.

Effect of Hydrogen Peroxide Destroying Agents on the Peroxide in Proskauer and Beck Types of Medium and on Growth of an Isoniazid-Resistant Strain of M. Tuberculosis

It was found that all the additives which promoted growth of isoniazid-resistant strains of *M. tuberculosis* in Proskauer and Beck types of medium were capable of destroying the peroxide in these media. However, for the most part this destruction took place rather slowly, so that, if a medium with a fairly high peroxide concentration was used, it was usually some days before the peroxide concentration was reduced to a level which could be expected to permit growth of these resistant strains.

In an attempt to show a correlation between the concentration of peroxide attained and the initiation of growth of a resistant strain, the two experiments summarized in table 4 were set up. A different medium was used in each experiment: one with a high peroxide concentration due to its manganese content (*Medium A*), and one with a low peroxide concentration (*Medium B*). The different additions were made at periods up to seven days before inoculation (only three periods are shown in the table). It should be made clear that the estimated concentrations of less than 1 γ of peroxide per ml. were approximate, especially when the addition was colored.

Because of the low peroxide concentration in *Medium B* all the additives except cysteine were able to reduce the level of peroxide to below 2 γ per ml. very rapidly and, consequently, all these permitted growth to take place. In the case of ascorbic acid and hemin, the peroxide was not brought below 2 γ per ml. at the time of inoculation of the zero-hour tubes, but destruction was taking place sufficiently rapidly (possibly accelerated in the incubator) for this to be accomplished in twenty-four hours or so. From results to be given later it is clear that the inoculum would not be sterilized in this time.

In the high peroxide medium the position was more complex. Quite a number of the additives succeeded in bringing the level of peroxide to about 2 γ per ml. or lower (though this usually required fairly prolonged exposure); however, most of them (0.1 per cent serum, hemin, Adams catalyst, charcoal, reductone, and ascorbic acid) did not permit growth. The probable explanation of this phenomenon is that new peroxide was being continuously formed during incubation as a result of the manganese present. This further peroxide was not removed by Adams catalyst or charcoal because these had been removed by filtration. Nor was it removed by reductone or ascorbic acid, because insufficient

TABLE 4

THE EFFECT OF ADDITIVES ON THE PEROXIDE CONCENTRATION AND ON GROWTH OF H37RvN IN PROSKAUER AND BECK MEDIUM

Addition	Concentration of Addition	*Medium A*—High Peroxide			*Medium B*—Low Peroxide		
		Days of Exposure Before Inoculation			Days of Exposure Before Inoculation		
		7	2	0	7	2	0
Serum	1%	0.0	0.0	0.7	0.9	0.7	0.5
		++	++	++	++	++	++
Serum	0.1%	1.1		7.8	0.4	0.5	0.8
		—	—	—	++	++	++
Bovine albumin fraction V	0.1%	9.6	9.6	9.6	0.8	1.1	1.2
		—	—	—	++	++	++
Hemin	2 γ/ml.	0.5	3.6	11.3	0.0	1.1	2.4
		—	—	—	++	++	++
Co^{++}	20 γ/ml.	0.0	2.4	9.5	0.0	0.0	0.8
		++	+	—	++	++	++
$FeCl_2$	100 γ/ml.	7.5	8.7	9.4			
		—	—	—	++	++	++
$FeSO_4$	100 γ/ml.	6.1	5.5	6.0			
		—	—	—	++	++	++
$KMnO_4$	10 γ/ml.	1.7	0.7	0.7	0.0	0.0	0.0
		—	—	—	—	—	—
Adams catalyst		2.1	2.3	10.7	0.9	0.9	0.8
		—	—	—	++	+	+
Activated carbon		0.5	6.0	9.8	0.3	0.3	
		—	—	—	++	++	+
Sodium hydrosulfite	50 γ/ml.	0.7	0.5	0.4	0.4	0.5	0.9
		++	++	++	++	++	++
Pyruvic acid	10 γ/ ml.	5.6	5.1		0.3	0.5	0.7
		—	—	—	++	++	++
Reductone	100 γ/ml.	0.9	1.2	12.1			
		—	—	—			
	20 γ/ml.				0.5	0.6	1.3
					++	++	++
	10 γ/ml.	8.1	8.1	11.2			
		—	—	—			
Ascorbic acid	100 γ/ml.	1.2	1.0	7.3			
		—	—	—			
	10 γ/ml.				0.9	0.5	2.5
					+	+	+
Glutamic acid	1 mg./ml.	9.8	9.8	10.1	0.9		0.9
		—	—	—	+	+	+
Cysteine HCl	10 γ/ml.	10.4	10.4	10.4	2.8		3.3
		—	—	—	±		±
Fructose	1 mg./ml.	9.9	9.9	9.6			
		—	—	—			
Vitamin B_{12}	100 γ/ml.	9.0		8.3			
		—		—	+		
Methylene blue	10 γ/ml.	9.3	10.8	10.7	0.9	1.1	1.0
		—	—	—	++	++	++
No addition		11.9	11.9	11.9	3.0	3.0	3.0
		—	—	—	—	—	—

The figures give the peroxide concentration in γ per ml. at the time of inoculation while the plus signs indicate relative amounts of growth after fourteen days.

All inocula were of the same suspension and were added to the tubes at the same time while peroxide determinations were being made on duplicate tubes.

amounts of these substances remained after their reaction with the initial peroxide, or by 0.1 per cent serum or hemin, because both of these were already inactivated in the course of the destruction of the initial peroxide.

These results then are all in keeping with the theory that the peroxide in the medium prevents the growth of isoniazid-resistant strains and that this peroxide must be reduced to the region of 2 γ per ml. or lower before growth of these strains can take place (from the usual inocula).

As has been pointed out elsewhere (14), strong evidence that the peroxide in the media is hydrogen peroxide was provided by experiments which showed that the peroxide destroying agents, listed in table 4 (varying widely in type and degree of activity) all removed this peroxide from the medium at the same rate as they removed added hydrogen peroxide.

Inhibitory Concentration of Hydrogen Peroxide for Isoniazid-Resistant Strains of M. Tuberculosis

Low-peroxide Proskauer and Beck medium was freed as far as possible from peroxide by shaking at intervals in a closed bottle with Adams catalyst (0.1 per cent) for two weeks. At the end of this period the peroxide content was below the detectable level (about 0.2 γ per ml.). Hydrogen peroxide was then added in a twofold dilution series and the tubes were inoculated immediately. The results for a number of strains are given in table 5.

It may be seen that, as other workers have found (4, 7), these highly isoniazid-

TABLE 5

INHIBITORY CONCENTRATION OF HYDROGEN PEROXIDE IN PROSKAUER AND BECK MEDIUM FOR DIFFERENT STRAINS OF M. TUBERCULOSIS

Concentration of H_2O_2 Added	Growth After Fourteen Days							
	H37Rv	BCG	H37RvN	Ravenel RvR	Hoey R	Flynn R	Cox R	Doyle R
γ *per ml.*								
64	−	−	−	−	−	−	−	−
32	+	±	−	−	−	−	−	−
16	3+	+	−	−	−	−	−	−
8	5+	2+	−	−	−	−	−	−
4	5+	3+	−	−	−	−	−	−
2	5+	5+	2+	±	−	−	−	−
1	5+	5+	4+	3+	±	±	±	±
0.5	5+	5+	4+	4+	+	+	+	+
0.25	5+	5+	4+	4+	+	+	+	+
0.12	5+	5+	5+	4+	2+	2+	2+	+
0.06	5+	5+	5+	4+	2+	2+	2+	+
0.03	5+	5+	5+	4+	2+	3+	3+	+
0	5+	5+	5+	4+	2+	3+	3+	+
5 per cent serum added	6+	6+	6+	6+	5+	5+	5+	5+

The growth was estimated on an arbitrary scale ranging from —, no growth, to 6+, which was heavy submerged growth.

resistant strains are all much more susceptible to peroxide in the growth medium than are drug-susceptible strains. The level of peroxide for complete inhibition of the resistant strains did not vary much from strain to strain, but there was a certain variation from experiment to experiment. These inconstancies seem to be due to variations in the inoculum and in the medium. For example, the minimal inhibitory concentration of hydrogen peroxide for H37RvN varied from 1.4–4.0 γ per ml.

In spite of the similar susceptibility of the different resistant strains to hydrogen peroxide, it can be seen from table 5 that there was a considerable difference in the rate of growth of these strains in media with less than 0.2 γ of peroxide per ml. These differences were almost eliminated by the addition of serum, suggesting that serum has some effect in addition to its peroxide destroying activity.

The fact that the inhibitory concentration of hydrogen peroxide for resistant strains is about the same as the inhibitory concentration of the peroxide formed in the medium is further evidence that this peroxide is hydrogen peroxide.

TABLE 6

BACTERICIDAL EFFECT OF HYDROGEN PEROXIDE ON H37RvN

Conditions and Length of Exposure to H_2O_2	Concentration of Hydrogen Peroxide		
	1.4 γ per Ml.	3.0 γ per Ml.	10.0 γ per Ml.
hours			
Medium at 37°C.			
0	6+	6+	6+
6	4+	4+	2+
12	4+	3+	±
24	3+	2+	0
48	2+	0	0
96	±	0	0
Medium at 0°C.			
0	6+	6+	6+
6	5+	5+	5+
12	5+	5+	4+
24	5+	5+	4+
48	5+	5+	4+
96	5+	5+	2+
Buffer at 37°C.			
0	6+	6+	6+
6	6+	6+	2+
12	6+	6+	±?
24	6+	6+	±?
48	6+	6+	±
96	6+	5+	+

Tubes containing the given concentration of H_2O_2 in Youmans media or phosphate buffer (pH 7) were inoculated as usual and incubated or refrigerated. After the appropriate period, 5 per cent serum was added to the tubes of medium; and the tubes of buffer were centrifuged, the supernatant removed, and the inoculum resuspended in Youmans medium containing 5 per cent serum. The figures give the density of growth on an arbitrary scale after fourteen days.

Bactericidal Effect of Hydrogen Peroxide on an Isoniazid-Resistant Strain of M. Tuberculosis

In order to determine the speed with which various concentrations of hydrogen peroxide killed the isoniazid-resistant strain, H37RvN, under various conditions, experiments such as those summarized in table 6 were carried out.

It may be seen that in Youmans medium at 37°C., ninety-six hours' exposure to 1.4 γ of hydrogen peroxide per ml. gave almost complete sterilization of the inoculum. This inoculum was rather more sensitive than that used in some other experiments (*see* table 5). Sterilization was completed by 3 γ per ml. in somewhat more than twenty-four hours, while with 10 γ per ml. sterilization was almost complete in twelve hours.

Keeping the temperature in the region of 0°C. during exposure to hydrogen peroxide prevented the latter from exerting its usual bactericidal effects. Partial protection was sustained against 10 γ per ml. for ninety-six hours. When buffer was substituted for medium during exposure to peroxide at 37°C., the cells were not killed at the lower concentrations, although little protection was given against 10 γ per ml.

The protective effect of low temperatures or lack of nutrients indicates that metabolizing cells are more sensitive to hydrogen peroxide than "resting" cells. A further experiment showed that the omission of asparagine alone from the medium did not protect against the effects of peroxide. This suggests that cell division is not necessary for high susceptibility to peroxide. The experiments were not conclusive, however, as there may have been sufficient traces of nitrogen from autolyzing cells and from medium carried over with the inoculum to permit some cell division.

Discussion

The results in this paper confirm the growing belief (3, 4, 7, 8) that the presence of peroxide in simple synthetic media is responsible for the failure of isoniazid-resistant strains of *M. tuberculosis* to grow in these media. The presence of peroxide in these media has been suspected for some time, but does not appear to have been detected by chemical methods hitherto.

The agents present in Proskauer and Beck medium which are responsible for this peroxide formation appear to be citrate, and traces of manganese in other constituents. The part played by manganese explains the finding (4) that manganese greatly increased the demand of isoniazid-resistant strains for peroxide destroyers. As manganese is effective in causing peroxide production in concentrations as low as 0.01 γ per ml., sufficient amounts of manganese are probably very frequently present in medium constituents. Such a concentration was found in the asparagine used in the media in this laboratory, while one batch of magnesium citrate contained enough manganese to introduce it into the medium at a final concentration of more than 1 γ per ml. From these experiments it appears that concentrations of peroxide in the region of 1 γ per ml. were likely to have been present in the media used by other workers.

In a previous paper (8) it was pointed out that the effects of various additives

on the growth of a number of isoniazid-resistant strains of *M. tuberculosis* in Proskauer and Beck medium were rather erratic. It has now been shown that, while all the additives which permit the growth of these strains are capable of destroying peroxide, they often do so at a slow rate so that the duration of exposure of the medium to the peroxide destroyer before inoculation is much more important than was previously realized. In the experiments described in the previous paper, the length of exposure to the peroxide destroyer was not standardized, and this led to variations in the amount of peroxide remaining at the time of inoculation.

It is probable that the difficulty of growing small inocula, even of isoniazid-susceptible strains of *M. tuberculosis* in synthetic media, unless certain additions (serum fractions (15), hemoglobin (16), or charcoal (17)) are made, is due at least in part to the peroxide present which these additives can destroy.

A discussion of medium constituents other than citrate and manganese which can give rise to peroxide production has been given elsewhere (14).

It appears that the peroxide formed in Proskauer and Beck medium and in some other media is hydrogen peroxide. In the former, it appears to be formed by the transfer, catalyzed by manganese, of hydrogen from citrate to molecular oxygen. Uri (18) has recently put forward a scheme for the formation of peroxide radicals from a reducing agent, oxygen, and metal ions such as Co^{++} or Mn^{++}, with the intermediate formation of a metal ion-oxygen complex.

Reducing agents may produce hydrogen peroxide by reduction of oxygen or may reduce hydrogen peroxide to water. In any given case the reaction which predominates will depend on a number of factors, including the nature and concentration of the reducing agent, the oxygen tension, the temperature, and the nature and concentration of the metallic ions present. Coleman and Middlebrook have recently published (19) strong inferential evidence that sulfhydryl compounds may act either as net peroxide formers or destroyers, depending on the concentration of these compounds and the oxygen tension.

It is clear from some of the results which have been obtained in the present study that serum has some stimulating effect on the growth of *M. tuberculosis* other than the destruction of peroxide. From the results in table 5 it may be seen that not only did serum stimulate growth beyond that obtained in the absence of peroxide, but also strains differing little in their reaction to peroxide were affected to very different degrees by the presence of serum. It was also noted that there was some variation in the growth obtained in different batches of medium with either the isoniazid-susceptible or -resistant strains—a variation which could not be correlated with the peroxide content of these media.

Summary

Chemical determinations have confirmed the presence of peroxide (very probably hydrogen peroxide) in Proskauer and Beck types of medium, usually at a concentration of 1–3 γ per ml.

The factors responsible for the formation of peroxide in these media have been investigated. It is mainly formed in the autoclave from citrate and traces

of manganese. Manganese can cause measurable peroxide formation at 0.01 γ per ml.

Hydrogen peroxide has been found to be very stable in these media.

All of the agents which promoted the growth of isoniazid-resistant strains have been shown to be capable of reducing the concentration of this peroxide, although often at a slow rate.

The minimal inhibitory concentration of hydrogen peroxide for the isoniazid-resistant strains studied was in the range 1.5–4 γ per ml.

The bactericidal effect of hydrogen peroxide for an isoniazid-resistant strain was investigated. "Resting" cells were found to be much less susceptible than metabolizing cells.

Sumario

Estudios Relativos a las Cepas Isoniacido-Resistentes del Mycobacterium Tuberculosis: III. El Efecto del Perόxido en los Medios

Las determinaciones químicas han confirmado la presencia de peróxido (muy probablemente de hidrógeno) en los medios de tipo Proskauer y Beck, por lo general a una concentración de 1–3 γ por ml.

Se han investigado los factores causantes de la formación de peróxido en estos medios. Fórmase principalmente en el autoclave de citrato y de indicios de manganeso. El manganeso puede provocar considerable formación de peróxido a 0.01 γ por ml.

El peróxido de hidrógeno ha resultado ser muy estable en estos medios. Todos los agentes que fomentaron la proliferación de cepas isoniacido-resistentes se mostraron capaces de reducir la concentración de este peróxido, aunque a menudo en baja proporción.

La mínima concentración inhibidora del peróxido de hidrógeno para las cepas isoniacido-resistentes estudiadas quedó dentro de los límites 1.5–4.0 γ por ml.

También se investigó el efecto bactericida del peróxido de hidrógeno para una cepa isoniacido-resistente. Las células "en reposo" se mostraron mucho menos susceptibles que las metabolizantes.

Resume

Recherches sur les souches isoniazido-résistantes du Mycobacterium-tuberculosis: III. Action du peroxyde dans les milieux

Des déterminations chimiques ont confirmé la présence de peroxyde (très probablement le peroxyde d'hydrogène) dans les milieux du type Proskauer et Beck, habituellement à la concentration de 1 à 3 γ par ml.

Les facteurs responsables de la formation de peroxyde dans ces milieux ont été recherchés. Le peroxyde est formé principalement dans l'autoclave à partir de citrate et de traces de manganèse. Le manganèse peut provoquer une formation appréciable de peroxyde lorsque sa concentration atteint 0,01 γ par ml.

Le peroxyde d'hydrogène a été trouvé très stable dans ces milieux. Tous les agents favorisant le développement des souches isoniazido-résistantes se sont montrés aptes à réduire la concentration de ce peroxyde, mais cette réaction est souvent lente.

La concentration inhibitrice minima du peroxyde d'hydrogène pour les souches isoniazido-résistantes étudiées variait entre 1,5 et 4 γ par ml.

L'action bactéricide du peroxyde d'hydrogène pour une souche isoniazido-résistante a été l'objet d'une investigation. Les cellules au repos (*resting cells*) ont été trouvées beaucoup moins sensibles que les cellules à métabolisme actif.

Acknowledgments

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REFERENCES

(1) Fisher, M. W.: The altered growth characteristics of isoniazid-resistant tubercle bacilli, Am. Rev. Tubere., 1952, *66*, 626.

(2) Cohn, M. L., Oda, U., Kovitz, C., and Middlebrook, G.: Studies on isoniazid and tubercle bacilli: I. The isolation of isoniazid-resistant mutants *in vitro*, Am. Rev. Tubere., 1954, *70*, 465.

(3) Cohn, M. L., Kovitz, C., Oda, U., and Middlebrook, G.: Studies on isoniazid and tubercle bacilli: II. The growth requirements, catalase activities, and pathogenic properties of isoniazid-resistant mutants, Am. Rev. Tubere., 1954, *70*, 641.

(4) Knox, R.: Haemin and isoniazid resistance of *Mycobacterium tuberculosis*, J. Gen. Microbiol., 1955, *12*, 191.

(5) Barry, V. C., Conalty, M. L., and Gaffney, E.: Isoniazid-resistant strains of *Mycobacterium tuberculosis*, Lancet, 1953, *1*, 978.

(6) Middlebrook, G.: Isoniazid resistance and catalase activity of tubercle bacilli, Am. Rev. Tubere., 1954, *69*, 472.

(7) Bönicke, R.: Die H_2O_2-Empfindlickheit INH-sensibler und -resistenter Tuberkelbakterienstämme, Naturwissenschaften, 1954, *41*, 577.

(8) Barry, V. C., Conalty, M. L., Denneny, J. M., Gaffney, E. E., and Winder, F.: Studies on isoniazid-resistant strains of *Mycobacterium tuberculosis*: I. Growth requirements of strains resistant to 100 micrograms of isoniazid per milliliter, Am. Rev. Tubere., 1955, *71*, 785.

(9) American Trudeau Society: Report of the Laboratory Subcommittee of the Committee on Medical Research and Therapy, Am. Rev. Tubere., 1950, *61*, 274.

(10) Youmans, G. P.: Improved method for testing of bacteriostatic agents using virulent human type tubercle bacilli, Proc. Soc. Exper. Biol. & Med., 1944, *57*, 119.

(11) Fisher, M. W.: Hemin as a growth factor for certain isoniazid-resistant strains of *Mycobacterium tuberculosis*, Am. Rev. Tubere., 1954, *69*, 797.

(12) Filomeni, M., and Siesto, A. J.: Micrometodo per la determinazione fotometrica di perossidi mediante la reazione con luminolo, Biol. soc. ital. biol. sper., 1951, *17*, 1096.

(13) Avery, O. T., and Morgan, H. I.: The occurrence of peroxide in cultures of pneumococcus, J. Exper. Med., 1924, *39*, 275.

(14) Barry, V. C., Conalty, M. L., Denneny, J. M., and Winder, F.: Peroxide formation in bacteriological media, Nature, 1956, *178*, 596.

(15) Dubos, R. J., and Davis, B. D.: Factors affecting growth of tubercle bacilli in liquid media, J. Exper. Med., 1946, *83*, 409.

(16) Frisch, A. W.: Multiplication of the H37Rv strain of *M. tuberculosis* on blood and its derivatives, Proc. Soc. Exper. Biol. & Med., 1952, *79*, 281.

(17) Hirsch, J. G.: Charcoal media for the cultivation of tubercle bacilli, Am. Rev. Tubere., 1954, *70*, 955.

(18) Uri, N.: Metal ion catalysis and polarity of environment in the aerobic oxidation of unsaturated fatty acids, Nature, 1956, *177*, 1177.

(19) Coleman, C. M., and Middlebrook, G.: The effects of some sulfhydryl compounds on growth of catalase-positive and catalase-negative tubercle bacilli, Am. Rev. Tubere., 1956, *74*, 42.

THE INFLUENCE OF THE STATE OF DISPERSION ON THE VIRULENCE OF SUSPENSIONS OF TUBERCLE BACILLI[1, 2]

HUBERT BLOCH[3] AND DENJI MIZUNO

(Received for publication July 9, 1956)

INTRODUCTION

This study is concerned with the relationship between the state of dispersion of bacterial suspensions used for infecting mice with tubercle bacilli and the virulence of such suspensions as measured by the survival times of mice. Since the early days of experimentation, workers have been aware of the possible role of bacterial clumps (1). Recently, Mayer and his collaborators (2) made a careful histopathologic investigation of the fate of bacterial clumps injected intravenously into mice. They found that bacterial suspensions prepared from ground surface-grown tubercle bacilli and containing aggregates measuring up to 10 by 20 μ in diameter were readily dispersed by leukocytes in the pulmonary capillaries. Injection of very heavy suspensions killed mice within a few minutes. The animals obviously died from embolism, and perhaps intoxication. When these suspensions were diluted, no immediate deaths were observed and the mice died with the symptoms of tuberculous pneumonia. Similar observations were also reported by Youmans and Youmans (3).

With the introduction by Dubos and his collaborators of liquid culture media in which mycobacteria grow submerged and diffused, it became possible to inject animals intravenously with the grown cultures in their original culture fluid. Yet, microscopic observation reveals that such cultures are by no means free of clumps and still contain aggregates probably consisting of hundreds of single bacteria (4). The size of these clumps varies with the bacterial strain, the age of the culture, and the concentration of Tween® 80 in the medium. Their influence on the virulence of the bacterial suspension has never been systematically studied. In a previous paper (5), a small experiment was reported in which the clump size of bacteria grown in Tween-albumin medium was altered by repeated washing in Tween-free saline. This procedure increased the average clump size, but seemingly did not alter the virulence of the culture. However, the experimental evidence was not convincing because such artificial clumps most likely differ from aggregates which are formed when the bacteria grow and multiply. They are probably less stable and more readily disrupted in the animal body than native bacterial aggregates.

Although bacterial aggregates probably do not play an important part under natural conditions and are an artifact of the experimental technique, the present

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results are reported because of the significant differences which were observed when mice were infected with clumped or completely dispersed cultures. Moreover, the question of the degree of clumping of a culture took on added significance in view of recent results (6) obtained when mice were infected with bacterial suspensions separated directly from tuberculous animals. Tubercle bacilli grown *in vivo* are almost always completely dispersed. Results of infection experiments with such bacteria are reported in the following paper (7).

Materials and Methods

Bacteria: Human tubercle bacilli of the strain H37Rv were grown in Tween-albumin medium containing 0.05 per cent Tween 80. All infection experiments were carried out with seven-day-old cultures. The native cultures consisted of a mixture of single cells and aggregates of from two to well over one hundred bacteria, arranged in characteristic serpentine cords. The virulence of these native cultures was compared with that of suspensions consisting of smaller units which were prepared in the following manner:

> The cultures were very gently shaken by hand for a few minutes with glass beads about 3 mm. in diameter. They were then filtered through paper (Whatman No. 12). Most of the larger aggregates were retained in the filter. The filtrates were then concentrated in the centrifuge and resuspended in the original culture medium to a suspension having the same turbidity as the original culture. The optical density was measured in a Coleman spectrophotometer.

The number of bacterial units in each suspension was determined by plate counts on oleic-acid-albumin agar and by direct counts using a Petroff-Hausser chamber. In the latter case, each bacterial unit was counted as one, whether it consisted of a single bacterial cell or of a smaller or larger clump. Since with the plate-count method every colony presumably emerges from one such unit, there should theoretically be perfect correlation between direct counts and colony counts. However, this was not always the case, and in some instances the direct counts were slightly higher than the colony counts. Thus, not every bacillus which was seen in a suspension always gave rise to a colony, perhaps because some cells were damaged or dead or because the agar contained some growth inhibitors. The differences were never significant and, in other instances, the plate counts gave higher numbers than the direct counts. Regardless of the method used, the filtered suspensions always gave counts about twenty to forty times higher than unfiltered cultures of the same age and equal optical density.

Mice: Male CF_1 mice (Carworth Farms, New City, New York), weighing about 20 gm. at the onset of the experiment, were used throughout. They were infected either intravenously with 0.1 ml. or intracerebrally with 0.03 ml. of bacterial suspension. The intracerebral injections were done in the usual way under light ether anesthesia. The mice were caged in groups of 10 to 20 and kept on a standard pellet diet. The length of their survival time after infection was used as a measure of virulence. Autopsies were performed on all dead mice.

Results

Virulence of Filtered and Unfiltered Bacterial Suspensions of Equal Turbidity upon Intracerebral Injection

Two experiments, *1A* and *1B*, were carried out in which the virulence of filtered and unfiltered suspensions of equal age and turbidity was compared in mice after intracerebral infection. The data pertaining to the bacterial preparations used in these experiments are summarized in table 1, and the survival curves of the infected mice are shown in figure 1*A* and *B*. Each experimental group consisted of 20 mice.

As may be seen in the figures, the unfiltered bacterial suspensions which consisted of fewer but larger bacterial units were considerably more virulent than the filtered ones which contained more but smaller units. In experiment *1A*, all mice injected with the unfiltered suspension died after twenty-eight days, while 70 per cent of the mice inoculated with the filtered suspension were still alive at that time. The experiment was discontinued after seventy days, at which time 20 per cent of the animals from the latter group were still alive. The second experiment, *1B*, gave similar results. After seventy days, 65 per cent of the mice injected with the filtered suspensions were still alive, whereas 90 per cent had died in the group which had received the unfiltered bacteria. Judging from turbidity measurements and dry weight determinations (not indicated in table 1), the bacterial masses compared in related experimental groups were identical.

Virulence of Filtered and Unfiltered Bacterial Suspensions upon Intravenous Injection

The data pertaining to this experiment (*Experiment 2*) are included in figure 2 and table 1. The same difference in virulence between the filtered and unfiltered bacterial suspensions was equally conspicuous with the intravenous route of infection. The experiment was repeated twice with identical results.

Virulence of Filtered and Unfiltered Bacterial Suspensions Consisting of Equal Numbers of Bacterial Units

In the preceding experiments, bacterial suspensions were used which consisted of approximately equal total numbers of bacteria, but which differed as to the distribution of the bacteria, i.e., the number (and size) of viable bacterial units. In the following experiment suspensions were used which had approximately equal numbers of units, but the units differed in size and hence the total bacterial mass was different. As could be expected, the unfiltered suspensions containing the larger units were much more virulent than the preparations consisting of equal but smaller numbers of bacterial units.

Experiment 3 of this design, in which mice were infected intracerebrally, is

TABLE 1

DATA PERTAINING TO THE NATURE OF THE BACTERIAL SUSPENSIONS USED IN THE EXPERIMENTS REFERRED TO IN THE TEXT

Experiment Number (Figure Number)	Treatment of the Bacterial Suspension	Number of Bacterial Units per Ml. (Direct Counts)	Turbidity of Final Suspension (Per Cent Light Transmittance)
1A	Filtered	245.3×10^6	38
1A	Unfiltered	7.5×10^6	38
1B	Filtered	248.0×10^6	48
1B	Unfiltered	13.5×10^6	48
2	Filtered	424.0×10^6	48
2	Unfiltered	16.5×10^6	49
3	Filtered	13.9×10^6	94
3	Unfiltered	13.5×10^6	47

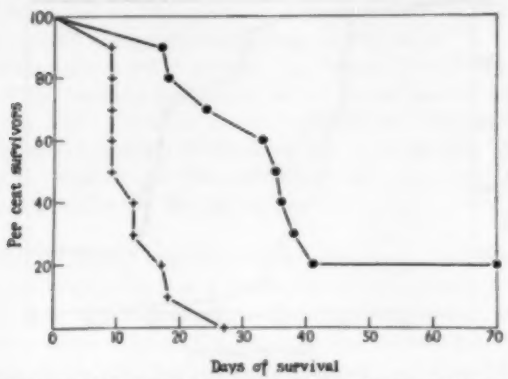

FIG. 1A. Survival of mice infected intracerebrally with suspensions of filtered and unfiltered tubercle bacilli. ●——● = filtered suspensions; +——+ = unfiltered suspensions. (For further explanations see text and table 1.)

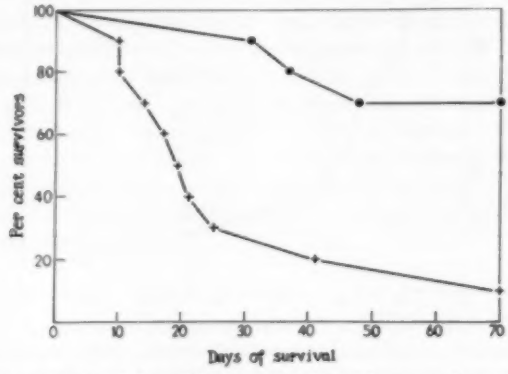

FIG. 1B. Survival of mice infected intracerebrally with suspensions of filtered and unfiltered tubercle bacilli. ●——● = filtered suspensions; +——+ = unfiltered suspensions. (For further explanations see text and table 1.)

summarized in figure 3 and table 1. Fifty per cent of the animals infected with the suspension consisting of the larger bacterial units died of tuberculosis within eighteen days after infection, whereas none of the mice infected with the same number of smaller aggregates died within the observation period of seventy days. When these animals were sacrificed and autopsies were performed at this point, they all had numerous fibrotic pulmonary lesions and greatly enlarged spleens containing large numbers of tubercle bacilli. Similar results were obtained with the intravenous route of infection. There, too, the unfiltered suspensions killed the mice within the observation period of two months, whereas none of the animals infected with the filtered cultures died of tuberculosis.

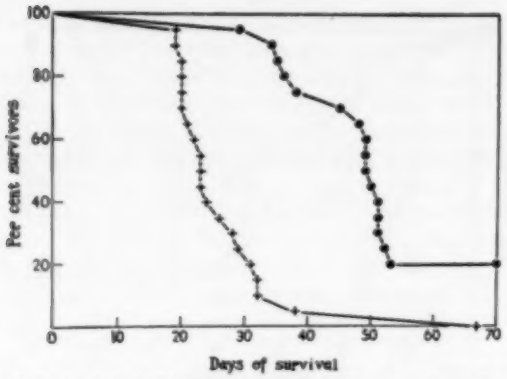

FIG. 2. Survival of mice infected intravenously with filtered and unfiltered suspensions of tubercle bacilli. Symbols are the same as in figure 1. (For further explanations see text and table 1.)

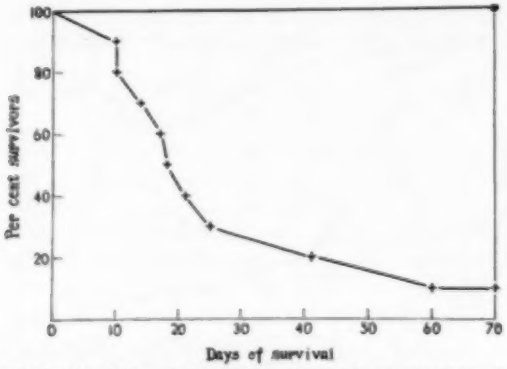

FIG. 3. Survival of mice infected intracerebrally with suspensions of filtered and unfiltered tubercle bacilli. Symbols are the same as in figure 1. (For further explanations see text and table 1.)

DISCUSSION

It is known that various mouse strains differ in their susceptibility to tuberculosis (8, 9) and also that the physiologic state of a bacterial culture may greatly influence its virulence for mice. But no quantitative information is available regarding the number of tubercle bacilli required to produce progressive lethal tuberculosis in the mouse. In other animal species, quantitative infection studies have been made. Lurie and his collaborators found (10) that in susceptible rabbits infected intra-alveolarly with virulent bovine tubercle bacilli each bacillus gave rise to a tubercle. When human bacilli were used instead, the number of "takes" was much smaller and many bacilli were destroyed before a tubercle was formed. As the natural resistance of the mouse to tuberculosis is relatively high,

it is likely that not all of the infecting organisms survive and that a certain number are destroyed during the early phase of infection.

Under these circumstances it seemed interesting to know whether the state of dispersion of the bacteria had any influence on the course of infection. Most investigators pay little attention to this variable; yet the average clump size varies from one bacterial strain to the other and is also greatly influenced by the composition of the medium. Even in media providing dispersed bacterial growth, the tubercle bacilli still grow in aggregates which often consist of hundreds of single bacilli.

The results of the present experiments indicate clearly that the average clump size of a bacterial suspension is of great significance in determining the progress of infection. Of two suspensions containing the same total number of bacteria from the same original culture, the one with the larger aggregates is also the more virulent for mice. This is true whether the mice are infected intracerebrally or intravenously. With the latter mode of infection, it could be argued that the injection of large aggregates is more severe because it causes pulmonary embolism. This argument is less likely to be valid in the case of intracerebral injections, especially since mice injected intracerebrally always die with the symptoms of extended pulmonary tuberculosis. Before reaching the lungs, most of the bacterial aggregates have probably been broken up into smaller units. As Mayer and associates (2) have shown, large bacterial clumps are readily disrupted by the action of leukocytes and, therefore, it is to be expected that the number of clumps in the lungs will be smaller on intracerebral than on intravenous injection. The most likely explanation for the greater virulence of bacterial suspensions consisting of larger units seems to be that the larger clumps offer more resistance to the early cellular reaction of the host and that a single bacillus is more readily destroyed by macrophages.

Summary

Pairs of suspensions were prepared from the same liquid culture of *M. tuberculosis* var. *hominis*, strain H37Rv, which contained an equal total number of bacteria but which differed in their state of dispersion. Groups of mice were infected with these bacterial preparations by the intravenous and the intracerebral routes. It was found that suspensions consisting of small bacterial units (single cells and small aggregates) were significantly less virulent than suspensions of comparable optical turbidity and dry weight, consisting of fewer but larger viable bacterial units. When suspensions were compared which contained comparable numbers of viable units, the preparations with the larger units were more virulent than the ones consisting of smaller bacterial aggregates.

Sumario

El Influjo del Estado de Dispersión sobre la Virulencia de las Suspensiones de Bacilos Tuberculosos

Se prepararon suspensiones en pares del mismo cultivo líquido del *M. tuberculosis* var. *hominis*, cepa H37Rv, que contenían un número total igual de bacterias, pero que discrepa-

ban en su estado de dispersión. Se infectaron grupos de ratones con esas preparaciones bacterianas por las vías endovenosa e intracerebral. Observóse que las suspensiones que constaban de pequeñas unidades bacterianas (células aisladas y pequeños agregados) eran decididamente menos virulentas que las suspensiones de turbidez óptica y peso seco comparables, que constaban de menos, pero mayores, unidades bacterianas viables. Al compararse suspensiones que contenían cantidades comparables de unidades viables, las preparaciones de las mayores unidades eran más virulentas que las compuestas de agregaciones bacterianas más pequeñas.

Resume

L'influence de l'état de suspension sur la virulence des suspensions de bacilles tuberculeux

Des suspensions en paires furent préparées à partir d'une même culture de *M. tuberculosis* var. *hominis*, souche H37Rv, elles contenaient un même nombre total de bactéries, mais différaient en ce qui concerne leur état de dispersion. Des groupes de souris furent inoculées avec ces préparations bactériennes par voies intraveineuse et intracérébrales. Il a été observé que les suspensions consistant en unités bactériennes petites (cellules uniques et agrégats réduits) présentaient une virulence notablement moindre que les suspensions de poids sec et de turbidité optique comparables, qui renfermaient des unités bactériennes viables plus volumineuses et moins nombreuses. Quand on comparait les suspensions qui renfermaient un nombre équivalent d'unités viables, les préparations renfermant les unités plus volumineuses étaient plus virulentes que celles composés d'agrégats bactériens plus petits.

REFERENCES

(1) Borrel, A.: Tuberculose pulmonaire expérimentale, Ann. Inst. Pasteur, 1893, *7*, 593.
(2) Mayer, E., Jackson, E. R., Whiteside, E. S., and Alverson, C.: Experimental embolic pulmonary tuberculosis in mice, Am. Rev. Tubere., 1954, *69*, 419.
(3) Youmans, G. P., and Youmans, A. S.: The relation between the size of the infecting dose of tubercle bacilli and the survival time of mice, Am. Rev. Tubere., 1951, *64*, 534.
(4) Fenner, F., and Leach, R. H.: The growth of mammalian tubercle bacilli in Tween-albumin liquid medium, Am. Rev. Tubere., 1953, *68*, 321.
(5) Bloch, H.: Studies on the virulence of tubercle bacilli: The relationship of the physiological state of the organisms to their pathogenicity, J. Exper. Med., 1950, *92*, 507.
(6) Segal, W., and Bloch, H.: Biochemical differentiation of *Mycobacterium tuberculosis* grown *in vivo* and *in vitro*, J. Bact., 1956, *72*, 132.
(7) Segal, W., and Bloch, H.: Pathogenic and immunogenic differentiation of *Mycobacterium tuberculosis* grown *in vitro* and *in vivo*, Am. Rev. Tubere., 1957, *75*, 495.
(8) Pierce, C., Dubos, R. J., and Middlebrook, G.: Infection of mice with mammalian tubercle bacilli grown in Tween-albumin liquid medium, J. Exper. Med., 1947, *86*, 159.
(9) Grumbach, A.: Der erbliche Einfluss auf Überlebensdauer, Organlokalisation und Manifestation im Tuberkuloseversuch, Schweiz. Ztschr. f. Path. u. Bakt., 1949, *12*, 614.
(10) Lurie, M. B., Heppleston, A. G., Abramson, S., and Swartz, I. B.: An evaluation of the method of quantitative airborne infection and its use in the study of the pathogenesis of tuberculosis, Am. Rev. Tubere., 1950, *61*, 765.

PATHOGENIC AND IMMUNOGENIC DIFFERENTIATION OF MYCOBACTERIUM TUBERCULOSIS GROWN IN VITRO AND IN VIVO[1, 2]

WILLIAM SEGAL AND HUBERT BLOCH

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INTRODUCTION

The development of a method for the separation of *Mycobacterium tuberculosis* in large numbers directly from the lungs of infected mice has enabled comparative studies of bacilli of the same strain grown *in vivo* and *in vitro*.

In a recent paper (1) the method has been described and striking differences have been reported for the biochemical activity of bacilli separated from infected mouse lungs (LRv) as compared with artificially cultured *M. tuberculosis*, strain H37Rv. However, no significant differences were found to exist when comparing tubercle bacilli, grown *in vivo* and *in vitro*, with respect to pathogenicity for mice. More recently it has been shown (2) that the degree of aggregation of H37Rv markedly affects its virulence for mice. When this factor was taken into account, it became apparent that LRv was considerably more virulent than H37Rv. Furthermore, significant immunogenic differences between H37Rv and LRv came to light.

MATERIAL AND METHODS

Bacterial strains: The source of tubercle bacilli grown *in vitro* was seven-day Tween®-albumin cultures of the virulent human strain, H37Rv, of *M. tuberculosis*.

The source of bacilli grown *in vivo* was the lungs of moribund or dead mice, eighteen to twenty-three days after injection. The bacilli were obtained according to the procedure described elsewhere (1). The method, briefly, consists in grinding up infected lungs in isotonic sucrose solution and separating the bacilli from the tissue debris by repeated fractionating centrifugation. The bacilli thus obtained are virtually free of any adhering host tissue and are viable as judged by plate-count and other methods. Initially, mice were infected with H37Rv, but thereafter with tubercle bacilli separated from infected lungs (LRv).

Preparation of bacterial suspensions: Suspensions of LRv and H37Rv were made in a salt solution containing 0.05 per cent Tween-80 and were adjusted to uniform optical density in the Coleman Universal spectrophotometer.

In order to obtain two preparations of H37Rv of decreasing degree of aggregation, the cultures were successively filtered through sterile Whatman No. 12 fluted filter paper and the filtrate was concentrated by centrifugation and resuspended to uniform optical density in Tween-salt solution.

Estimations of the quantity of bacteria present in the various suspensions of equal optical density were made in several ways. Viability was determined by plate counts on oleic-acid-albumin agar. The total bacterial mass was determined by dry weight of distilled-water-washed bacilli. Estimation of the degree of aggregation was obtained by direct microscopic

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count of smears of 0.05 ml. in an area of 1 sq. cm. stained by the Ziehl-Neelsen method. Counts were made in three representative microscopic fields and the average value was used.

Virulence for mice: Male CF_1 mice (Carworth Farms, New City, New York) were injected intravenously with 0.1 ml. of bacterial suspensions adjusted to equal optical density. Groups of 15 mice each were used. The length of their survival time after infection served as a measure of virulence.

Immunity in mice: The vaccines of H37Rv and LRv were prepared by phenol treatment as described in a previous publication (3). Plate counts showed that this treatment resulted in complete killing of all bacilli for both vaccines. Groups of 15 mice each were injected intravenously with 0.1 ml. of LRv and H37Rv, equal to 0.5 mg. dry weight of bacilli. After three weeks the mice were challenged by intravenous injection of either a seven-day Tween culture of H37Rv or of a suspension of LRv separated on the same day from lungs of infected mice. The degree of immunity was measured by a comparison of the survival times of vaccinated mice and nonvaccinated control mice.

Results

Comparative Virulence for Mice of Lung Bacilli and Artificially Cultured Bacilli

A quantitative comparison of unfiltered suspensions of LRv with unfiltered and filtered suspensions of H37Rv is shown in table 1. Adjustment of all four suspensions to standard optical density resulted in equal concentrations of total bacillary mass as indicated by dry weight and by the total number of bacilli per microscopic field. The values obtained from plate counts reflect the degree of aggregation of the various suspensions, which is also indicated by the number of aggregates per microscopic field. The percentage of total bacilli appearing in different sizes of aggregates per microscopic field is shown in table 2. In the unfiltered suspensions of LRv, all of the bacilli appear in clumps of less than 30, with the highest concentration in groups of 2–10. This does not necessarily reflect the state of aggregation in the lung, but rather the method of separation of bacilli from lung tissue by differential centrifugation. Unfiltered Tween cultures of H37Rv are seen to exist in highest concentration in groups of approximately 200 bacilli, due to characteristic cord formation. After primary filtration, the

TABLE 1

Quantitative Comparison of Unfiltered Suspensions of Lung Bacilli (LRv) with Unfiltered and Filtered Suspensions of H37Rv*

Type	Number of Aggregates per Microscopic Field†	Total Number of Bacilli per Microscopic Field†	Plate Count per Ml.	Dry Weight per Ml. Mg.
Unfiltered LRv	332	2,181	42×10^6	0.21
Unfiltered H37Rv	74	2,088	9×10^6	0.23
Once filtered H37Rv	175	2,207	26×10^6	0.22
Twice filtered H37Rv	278	2,125	75×10^6	0.26

* All suspensions were adjusted to optical density 0.4 at 550λ in a Coleman Universal Spectrophotometer.

† Average of 3 representative microscopic fields in a smear of 0.05 ml. in 1 sq. cm. Values multiplied by a microscopic factor of 100,000 give approximate amounts per ml.

TABLE 2

COMPARISON OF DEGREE OF AGGREGATION OF UNFILTERED SUSPENSIONS OF LUNG BACILLI (LRv) WITH UNFILTERED AND FILTERED SUSPENSIONS OF H37Rv*

Percentage of Total Bacilli Appearing in Various Sizes of Aggregates per Microscopic Field†

Type	Single Bacilli	Clumps of 2–10	Clumps of 11–20	Clumps of 21–30	Clumps of 31–40	Clumps of 41–50	Clumps of More than 50
Unfiltered LRv	3.6	55.8	26.8	13.8	0	0	0
Unfiltered H37Rv	1.2	6.3	8.6	7.2	0	0	76.7
Once filtered H37Rv	4.0	4.9	12.9	15.9	31.7	30.6	0
Twice filtered H37Rv	7.1	13.8	32.5	20.0	18.1	8.5	0

* All suspensions were adjusted to optical density 0.4 at 550λ in a Coleman Universal Spectrophotometer.

† Average of 3 representative microscopic fields in a smear of 0.05 ml. in 1 sq. cm. Values multiplied by a microscopic factor of 100,000 give approximate amounts per ml.

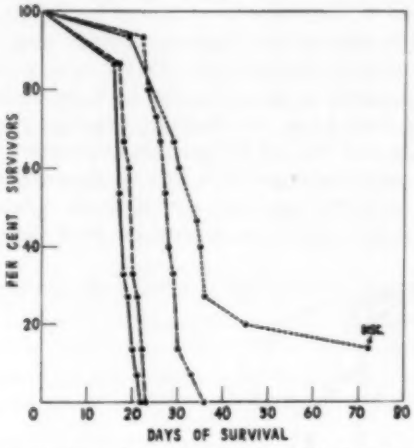

FIG. 1. Comparative virulence for mice of lung bacilli (LRv) and artificially cultured bacilli (H37Rv) with degrees of aggregation approaching that of LRv.

●——● LRv unfiltered
●— — —● H37Rv unfiltered
●— - — -● H37Rv filtered once
●·····● H37Rv filtered twice

large aggregates of more than 50 bacilli are eliminated in the H37Rv suspensions, and the highest concentration of bacilli is in the 31–40 and 41–50 groups. After the second filtration, the distribution of the bacilli is concentrated in the 11–20 and 21–30 groups, but 8.5 per cent of the bacilli are still in the 41–50 group. Thus, even after two filtrations, the H37Rv suspension was still more aggregated than the unfiltered LRv. Suspensions of LRv were not filtered because it was found that they were retained to a large extent by the filter paper, which would have necessitated the sacrifice of extremely large numbers of mice.

The comparative virulence for mice of lung bacilli and culture-grown bacilli with approaching degrees of aggregation is shown in figure 1. The curve of per cent survival of mice infected with unfiltered H37Rv was always similar to that shown for LRv. After one filtration, H37Rv showed a marked reduction in the degree of virulence. After the second filtration, the reduction was even greater, with 13.3 per cent survival of mice after seventy-two days, at which time the experiment was discontinued. It is to be expected from these results that, on further filtration of H37Rv resulting in a suspension of an equal state of aggregation as for LRv, the observed difference in virulence will be emphasized still further.

Comparative Degrees of Immunity Produced in Mice by Vaccines of Lung Bacilli and Artificially Cultured Bacilli

As seen in figure 2, phenol-killed H37Rv vaccine proved to be more effective immunologically than similarly prepared vaccine of LRv. An explanation for the observed difference in the immunity produced by H37Rv in mice might be the fact that the bacilli used for the challenge infection were H37Rv. However, even when LRv was used as the challenge, H37Rv vaccine continued to confer a greater degree of immunity to mice than did LRv (figure 2). It is further apparent from figure 2 that, when the challenge infection was carried out with H37Rv, both the LRv and the H37Rv vaccines gave better relative protection than when the mice were challenged with LRv. It thus appears that it is more difficult to produce protection against tubercle bacilli obtained from lungs of nfected mice than against artificially cultured bacilli of the same strain.

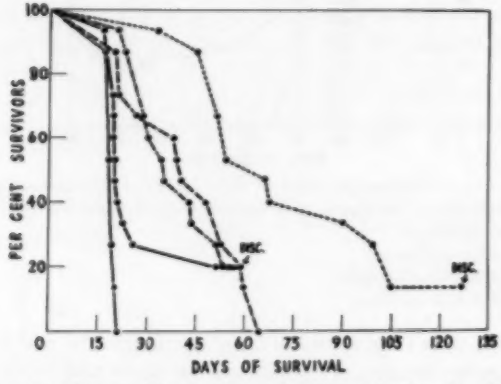

FIG. 2. Comparative degree of immunity produced in mice by phenol-killed vaccines of lung bacilli (LRv) and artificially cultured bacilli (H37Rv).

● ~~~~~~ ● Infection control (similar for H37Rv and LRv)
● ——— ● LRv vaccine, LRv challenge infection
● – – – – ● H37Rv vaccine, LRv challenge infection
● — — — ● LRv vaccine, H37Rv challenge infection
● ····· ● H37Rv vaccine, H37Rv challenge infection

Discussion

The pronounced difference in virulence and in immunizing powers of tubercle bacilli separated from infected mouse lungs and bacilli of the same strain grown in artificial culture medium justifies the conclusions drawn in a previous paper (1) on the basis of biochemical differences, namely, that LRv and H37Rv are dissimilar and can be regarded as strain variants of common heritage. As stated in that paper, the differences between them are likely to be adaptive in nature, as evidenced by the biochemical reversion of LRv to H37Rv after primary growth with a prolonged lag phase in artificial culture medium. Similarly, when H37Rv is used to infect mice it reverts in the animal body to the pathogenic strain of its origin. The lower degree of virulence of H37Rv for mice, compared with LRv, can thus be interpreted as due to the necessity to adapt to a highly different environment *in vivo*. On this assumption, the conversion of H37Rv to LRv should theoretically also be possible *in vitro*, if the essential environmental conditions determining the dissimilar characteristics of LRv *in vivo* were provided in artificial culture medium.

The lesser degree of immunity induced by a phenol-killed vaccine of LRv than by a similar vaccine of H37Rv is surprising since an LRv vaccine would be expected to offer better protection against homologous infection. The fact that it does not do so is not necessarily an indication that LRv is antigenically less complete than H37Rv. Other factors which are known to play a role in immunization may be involved. LRv may well be poorer in constituents with adjuvant activity than H37Rv. The ease with which LRv suspends in water may indicate a deficiency in lipid content as compared with H37Rv. This has actually been borne out by preliminary findings which indicate that LRv contains less chloroform-soluble lipids than H37Rv (4). Another factor which might contribute to the reduced immunogenicity of LRv vaccine is phenol treatment, which may affect LRv differently from H37Rv. Regardless of the explanation, the fact than an immunologic difference has been found to exist, further serves to establish the difference between tubercle bacilli cultured *in vivo* and those cultured *in vitro*.

The pronounced differences observed in virulence and in degree of immunizing ability between tubercle bacilli grown in the lung and artificially cultured tubercle bacilli of the same strain corroborate the biochemical differences reported in a previous paper (1). These differences, considered together, firmly establish the fact that tubercle bacilli in their natural infectious state are dissimilar to those grown in artificial culture medium.

Summary

The pathogenicity and immunogenicity of suspensions of tubercle bacilli, separated by a method of differential centrifugation from the lungs of tuberculous mice, were compared in mouse experiments with suspensions of the same virulent human strain of tubercle bacilli cultured in Tween®-albumin medium. The bacteria grown *in vivo* proved to be more virulent for mice than equal suspensions of bacilli grown *in vitro* with a similar degree of aggregation. Phenol-

killed vaccines prepared from tubercle bacilli grown *in vitro* produced a greater degree of immunity against tuberculosis in mice than comparable vaccines from bacilli grown *in vivo*. This was true whether the immunized mice were challenged with tubercle bacilli grown *in vitro* or *in vivo*. These results, considered together with biochemical differences previously reported, establish the fact that tubercle bacilli in their natural infectious state are dissimilar to bacilli of the same strain grown in artificial culture medium.

Sumario

Diferenciación Patógena e Inmunógena del Mycobacterium Tuberculosis Cultivado In Vitro e In Vivo

En experimentos en ratones compararónse la patogenicidad y la inmunogenicidad de suspensiones de bacilos tuberculosos, separados por un método de centrifugación diferencial de los pulmones de ratones tuberculosos, con las de suspensiones de la misma cepa humana virulenta de bacilos tuberculosos cultivados en el medio de Tween K- albúmina. Las bacterias cultivadas *in vivo* resultaron ser más virulentas para los ratones que suspensiones iguales de bacilos cultivados *in vitro* con un grado semejante de agregación. Las vacunas preparadas de bacilos tuberculosos cultivados *in vitro* y matadas con fenol produjeron mayor inmunidad contra la tuberculosis que vacunas comparables preparadas de bacilos cultivados *in vivo*. Esto fué un hecho independientemente de si los ratones inmunizados eran provocados con bacilos tuberculosos cultivados *in vitro* o *in vivo*. Estos resultados, considerados junto con las diferencias bioquímicas expuestas anteriormente, establecen firmemente el hecho de que los bacilos tuberculosos en su estado infeccioso natural son desemejantes de los de la misma cepa cultivada en un medio artificial.

Resume

Différenciation du pouvoir pathogène et immunogène du Myocbacterium tuberculosis développé in vitro ou in vivo

L'activité pathogène et immunogène de suspensions de bacilles tuberculeux séparés par une méthode de centrifugation sélective à partir de poumons de souris tuberculeuses, fut comparée dans une expérimentation sur la souris avec la même souche virulente de bacilles tuberculeux cultivée en milieu Tween-albumine. Les bacilles développés *in vivo* ont démontré, chez la souris, une virulence plus grande que des suspensions équivalentes de bacilles développés *in vitro* et dont les particules avaient un degré d'agrégation similaire. Les vaccins tués par le phénol et préparés à partir de bacilles tuberculeux développés *in vitro* produisaient un degré d'immunité antituberculeuse plus élevé chez les souris que les vaccins comparables provenant de bacilles développés *in vivo*. Il en était ainsi, que la souris immunisée ait été soumise à l'inoculation de bacille tuberculeux développés *in vitro* ou *in vivo*. Ces résultats, confrontés avec les différences biochimiques antérieurement rapportées, confirment le fait que les bacilles tuberculeux à l'état d'infection naturelle diffèrent des bacilles de la même souche cultivés dans les milieux artificiels.

REFERENCES

(1) Segal, W., and Bloch, H.: Biochemical differentiation of *Mycobacterium tuberculosis* grown *in vivo* and *in vitro*, J. Bact., 1956, *72*, 132.

(2) Bloch, H., and Mizuno, D.: The influence of the state of dispersion on the virulence of suspensions of tubercle bacilli, Am. Rev. Tuberc., 1957, *75*, 488.

(3) Bloch, H., and Segal, W.: Viability and multiplication of vaccines in immunization against tuberculosis, Am. Rev. Tuberc., 1955, *71*, 228.

(4) Unpublished observations.

Editorial

Treatment of Female Genital Tuberculosis

In a recent article in THE AMERICAN REVIEW OF TUBERCULOSIS AND PULMONARY DISEASES on the treatment of tuberculosis, two sentences were devoted to the treatment of female genital tuberculosis. While little has been written on this subject in journals of internal medicine, a large number of articles have recently appeared in gynecologic and surgical journals.[1] The purpose of this editorial is to review some of these papers and to present our own concepts of the current treatment of female genital tuberculosis.

The following methods of treatment of genital tuberculosis are now available: (*1*) hospital or sanatorium care; (*2*) antimicrobial therapy; (*3*) surgery and antimicrobial therapy. Roentgen-ray therapy and radium, although used in the past, no longer have a place in the treatment of pelvic tuberculosis.

Many of the cures of genital tuberculosis claimed in the past for hospital or sanatorium treatment alone must be regarded as unproved. Frequently the diagnosis was never confirmed by bacteriologic or pathologic means and the patient was treated solely on the basis of presumptive signs or symptoms of genital tuberculosis. The writer agrees that bed rest is essential in certain stages of the treatment of genital tuberculosis, namely, during an acute episode or in the presence of fever, pain, or secondary infection. It is believed, however, that bed rest alone is not the treatment of choice for genital tuberculosis and that more active measures (which will be discussed below) in association with bed rest are necessary to effect a cure.

This point of view is borne out by the report of Sutherland.[2] In a group of 109 patients, 57 patients chosen at random were treated with antimicrobial therapy, and the remaining 52 were allocated to a control group. Follow-up information was available for 49 patients in the treated group. Of 27 patients followed for at least one year after cessation of treatment, 3 showed evidence of recurrence of disease. The other 18 patients have been followed for less than one year and thus far none has shown evidence of recurrence. Follow-up data were available in 49 patients in the control group. Ten patients had to be removed from this group because of clinical deterioration which required immediate treatment. Of 25 patients followed for at least one year, 20 still showed evidence of active tuberculous endometritis. Of 14 patients followed for less than one year, 8 had evidence of active disease in the endometrium, while 6 had no evidence of activity.

Before undertaking antimicrobial therapy for female genital tuberculosis, one should determine, if possible, whether long-term chemotherapy by itself is contemplated or whether surgical intervention after a course of antituberculous

[1] A report on this subject by the Committee on Therapy of the American Trudeau Society appears on page 524 in this issue of the REVIEW.

[2] SUTHERLAND, A. M.: J. Obst. & Gynaec. Brit. Emp., 1954, *61*, 614.

drugs is to be carried out. To make this decision, the extent of involvement of the pelvic organs must be estimated. For this purpose the present writer has suggested a simple classification based on clinical evaluation of the extent of involvement of the fallopian tubes. Two types of pelvic lesion are differentiated, advanced and minimal.

Advanced pelvic tuberculosis is that in which endometrial tuberculosis is demonstrated by bacteriologic or microscopic examination. In addition there are palpable masses in the adnexa which are presumed to be tuberculous. This presumption is based on the knowledge that tuberculous endometritis is practically always secondary to tuberculous salpingitis.

Minimal pelvic tuberculosis is that discovered in the endometrium by bacteriologic or microscopic methods. It is symptomless and there is no apparent evidence of tuberculosis elsewhere in the genital tract. Careful pelvic examination fails to reveal evidence of tubal enlargement. This type of minimal disease has also been described as unsuspected or latent tuberculosis. There undoubtedly is an intermediate stage between the advanced and minimal types of pelvic tuberculosis, but this is not apparent on clinical examination.

A number of investigators have attempted to eradicate pelvic tuberculosis completely by the use of chemotherapy. The following regimens have been used:

Streptomycin alone. A dose of 1 gm. daily for three months was used. In a series of 184 patients collected from the literature treated by this method who were followed from four months to two years, only 52 per cent of the patients were improved.[2-5]

Streptomycin and PAS. The average doses employed were 1 gm. of streptomycin daily and 12 gm. of PAS daily. A group of 162 patients treated in this manner for an average of three months was assessed after two years. In this series 75 per cent of the patients were improved.[2, 6, 7]

Isoniazid alone. A group of 32 patients was treated with isoniazid alone, using 150 mg. per day, for four months. Follow-up information is available on this group for eight months and shows 91 per cent of the patients improved.[8]

Isoniazid, streptomycin, and PAS. Twenty-nine patients received isoniazid, streptomycin, and PAS for four months to one year or longer. Follow-up information on 18 of these patients for at least one year shows all but one patient improved.[7, 9, 10]

Difficulties arise in evaluating the efficacy of antimicrobial therapy alone in the treatment of advanced female genital tuberculosis. One of the problems is to determine whether and when the pelvic tuberculosis has been cured by antimicrobial therapy. The finding of a nontuberculous endometritis on bacteriologic

[2] LEROUX, M., AND GUIHENEUC, B. O.: Gynec. et obst., 1950, *49*, 479.
[4] RYDEN, A. B. V.: Acta obst. et gynec. Scandinav., 1953, *32*, 368.
[5] ZAMORANO, L. B.: Bol. Soc. chilena de obst. & ginec., 1951, *16*, 17.
[6] MILLAR, W. G.: J. Obst. & Gynaec. Brit. Emp., 1954, *61*, 372.
[7] STALLWORTHY, J. A.: Personal communication.
[8] BARNS, T., *et al.*: Lancet, 1953, *1*, 817.
[9] SCHAEFER, G.: Am. J. Obst. & Gynec., 1955, *69*, 1333.
[10] SNAITH, L. M.: Personal communication.

and microscopic examination is not sufficient evidence of cure since tuberculous salpingitis has been shown to persist despite the healing of a coexistent tuberculous endometritis.[11] Definite healing can be determined only if the pelvic organs are available for examination. In those instances in which surgical removal of fallopian tubes has been performed after antimicrobial therapy, active tuberculous salpingitis persisted in some and, in those tubes apparently healed, microscopic examination revealed them to be so disorganized as to render them functionless.

Of the four regimens cited above, adequate antimicrobial therapy, by present standards, was employed only in *Group 4*. In order to determine the results of longer periods of antimicrobial therapy in minimal pelvic tuberculosis, the writer has employed the following regimen. The patient is given a course of isoniazid and PAS as follows:

> Isoniazid, 5 mg. per kg. of body weight daily, orally (usually 300 mg. per day) plus 12 gm. of PAS orally every day for one year or longer. If PAS cannot be tolerated, streptomycin, 1 gm. twice a week, is given. Four months after cessation of treatment, bacteriologic and microscopic examinations of endometrial curettings and secretions are done. These examinations should be repeated every three months for several years. If they are negative for evidence of tuberculous infection, a Rubin test or a hysterosalpingogram is made the following month. If there is tubal patency, some hope for conception exists, although mere patency is not proof of tubal function that would enable passage of a fertilized ovum.

In none of the writer's cases has pregnancy resulted thus far. Millar was able to demonstrate tubal patency after a course of antimicrobial therapy in three of nineteen patients in whom apparent tubal blockage had existed prior to treatment. None of the patients in the present study had palpable adnexal masses before treatment and in none has pregnancy been reported thus far.

Even with continuous chemotherapy, however, local spread of minimal pelvic tuberculosis has been reported. Brown and associates[12] cite a patient in whom pelvic tuberculosis was diagnosed by endometrial curettage as part of a sterility study. The patient was otherwise asymptomatic and no adnexal masses were palpable. While receiving isoniazid, streptomycin, and PAS, she developed bilateral adnexal masses which at laparotomy were found to be tuberculous.

At the present time, there would appear to be no valid reason to perform surgery in a woman known to have genital tuberculosis without a preoperative course of antituberculous drugs. While the optimal duration of preoperative drug therapy has not been determined, on the basis of the experience to date it should be at least three months. The duration of postoperative drug therapy will depend on whether all tuberculous foci in the pelvis are removed as well as on the presence of extra-pelvic tuberculosis. At present, it is the writer's practice to give antimicrobial therapy for twelve months after operation. Postoperatively the pa-

[11] O'Driscoll, D. T.: Lancet, 1951, *2*, 476.
[12] Brown, A. B., *et al.*: Obst. & Gynec., 1953, *2*, 476.

tient's activity is gradually increased for three months, after which full activity is allowed.

There are occasions, however, when operation will be performed in patients in whom a diagnosis of pelvic tuberculosis was not made preoperatively or even at the time of operation.

When unsuspected genital tuberculosis is discovered at the operating table, in the presence of extensive disease a bilateral salpingo-oophorectomy and hysterectomy should be performed. If such a procedure cannot be carried out because of technical difficulties, the abdomen should be closed without drainage and a course of antituberculous drugs should be given for three to four months. Repeated pelvic examinations will usually show sufficient regression of the disease to enable operative removal of the diseased organs at a later date. Postoperative antituberculous therapy should be given for at least twelve months.

Occasionally the microscopic examination of a pelvic organ removed at operation reveals unsuspected tuberculosis. If inadequate surgery, such as salpingostomy, unilateral salpingectomy, or subtotal hysterectomy, has been performed, the patient should receive a course of antituberculous therapy and be reoperated upon. If all the diseased organs were removed at the original operation, then a course of antituberculous drugs should be given postoperatively and a careful check made for other foci of tuberculosis.

The minimal operative procedure that should be performed in the presence of tuberculous salpingitis is bilateral salpingectomy. If plastic operations on the fallopian tubes have been performed for the cure of infertility and tuberculous salpingitis is discovered in the portion of tube removed, one is faced with a difficult problem. The patient should immediately be given isoniazid and an auxiliary drug, for the danger of miliary tuberculosis or hematogenous spread exists. Antituberculous therapy should be continued for at least one year. If it is then thought that the danger of an exacerbation no longer exists, the patency of the tubes can be tested. The patient must be kept under constant surveillance and repeated investigations for the presence of pelvic tuberculosis made.

Before the use of antituberculous drugs preoperatively in the management of female genital tuberculosis, better results were obtained following radical procedures than following conservative operations. Removal of all tuberculous foci resulted in more prompt healing than when conservative procedures were performed, with the retention of an organ or part of an organ containing tuberculosis. Recurrences, fistulas, and spread to distant areas in the body were described following inadequate or incomplete surgery.

Pelvic organs, i.e., the uterus, tubes, or ovaries, that appear on gross examination to be involved by tuberculosis should be removed. The writer believes that the tubes are involved first in genital tuberculosis and that they serve as a focus of infection for the uterus and ovaries. Therefore, the minimal surgery that should be performed is bilateral salpingectomy. However, the disease in all patients with genital tuberculosis is not diagnosed early, and other factors, which will be discussed below, enter into the decision to perform radical or conservative surgery.

Many operators believe that the procedure of choice for advanced pelvic tuberculosis is total hysterectomy and bilateral salpingo-oophorectomy. In a patient forty years of age or older, this type of surgery would not be considered radical. However, the same procedure performed for similar disease in a woman in her early twenties has been questioned. The rationale for removal of the uterus, tubes, and ovaries is that, since the tubes are involved in almost all cases of female genital tuberculosis, the uterus in more than half of the patients, and the ovaries in about one-fourth of the patients, extirpation of these organs removes the tuberculous foci completely. If the ovaries do not appear involved in a very young patient, they need not be removed. The ovaries should be bisected and carefully inspected for areas of tuberculosis which may be present in the hilus. There have been occasional cases in which a caseous focus was present in the hilus of the ovary which subsequently went on to necrosis of the entire organ and resulted in miliary tuberculosis.

There are some who believe that the use of antimicrobial therapy will result in more conservative operative procedures for genital tuberculosis. The greatest difference of opinion exists as to whether the uterus and the ovaries should be removed in addition to the tubes. If recurrence of tuberculosis in the endometrium is noted after a course of chemotherapy, it would appear inadvisable to retain the uterus. If repeated microscopic and bacteriologic examinations of uterine contents reveal no evidence of tuberculosis and if, at operation, only the tubes and not the ovaries are involved, and if all this occurs in a young patient who is desirous of retaining her menstrual function, conservative surgery might be attempted.

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Notes

THE SIGNIFICANCE OF DELAYED EMERGENCE OF GROWTH OF TUBERCLE BACILLI[1]

Reports of emergence of growth of tubercle bacilli after periods of incubation greater than the six to eight weeks usually allowed have recently become more common.[2-5] In general these reports have referred to patients who were receiving or had previously received chemotherapy, and the delay in emergence of growth has been attributed to the damage done to the organisms, their depressed metabolic state presumably resulting from exposure to the drugs.

Results reported in this paper do not support that point of view. Rather, it is believed that the media previously used for isolation purposes have not only not been optimal for the conditions studied, as was assumed, but they have been inadequate in that they failed to contain agents able to counteract effectively the inhibitory effects of the drugs being used to treat the patients.

Of the three drugs, streptomycin, isoniazid, and para-aminosalicylic acid (PAS), PAS has been shown to exert the most marked suppressive action[6, 7]; isoniazid some but less; and streptomycin, little or none under the conditions tested. Medium containing para-aminobenzoic acid (PABA) has been shown capable of antagonizing this inhibitory effect.[6, 8]

In this study, sputum specimens were obtained from a series of patients who had received for five to eighteen weeks initial courses of chemotherapy consisting of PAS along with either streptomycin or isoniazid and continued to receive therapy during the collection period. Specimens were digested, concentrated, neutralized, and the sediments were suspended in buffered 0.1 per cent fraction V bovine albumin. The suspensions were mixed thoroughly, and an equal volume was pipetted onto the surface of each tube of medium used, in a staggered, randomized order. Only solid media in screw-capped tubes were used and, after the excess moisture had evaporated (usually one week after inoculation), the caps, left loose until then, were tightened to prevent drying. They were not thereafter loosened except to remove portions of colonies grossly typical or suggestive of tubercle bacilli for staining or subculture.

Each specimen was inoculated on duplicate tubes of Löwenstein-Jensen egg medium,[9] duplicate tubes of blood agar[10] containing 100 units of penicillin per ml., duplicate tubes of oleic-acid-albumin agar,[11] containing 75 units of penicillin per ml., the latter medium enriched by the addition of 2 γ of biotin per ml. and 5 γ of pyridoxine hydrochloride per ml. (hereafter called enriched medium), and the enriched medium plus varying quantities of

[1] From the Research Laboratory, Veterans Administration Hospital, Alexandria, Louisiana.

[2] Tarshis, M. S.: Tr. Eleventh Conference on the Chemotherapy of Tuberculosis, Veterans Administration, Army, and Navy, 1952, p. 237.

[3] Howard, O. P., *et al.*: Am. Rev. Tuberc., 1954, *69*, 307.

[4] Hobby, G. L., *et al.*: Am. Rev. Tuberc., 1954, *70*, 191.

[5] Jones, A. W., and Gentry, W. H.: Am. Rev. Tuberc., 1955, *71*, 319.

[6] Yegian, D., *et al.*: Am. Rev. Tuberc., 1955, *71*, 860.

[7] Duerr, E. L., and Black, T. C.: Dis. of Chest, 1956, *30*, 306.

[8] Duerr, E. L.: Bact. Proc., 1955, p. 98.

[9] Holm, J., and Lester, V.: Pub. Health Rep., 1947, *62*, 847.

[10] Tarshis, M. S.: J. Lab. & Clin. Med., 1952, *40*, 628.

[11] Dubos, R. J., and Middlebrook, G.: Am. Rev. Tuberc., 1947, *56*, 334.

TABLE 1

AMOUNT OF GROWTH* OF M. TUBERCULOSIS FROM SELECTED SPUTUM SPECIMENS

Type of Medium†	No. 466, Patient FH Isoniazid and PAS (5 weeks)					No. 424, Patient WA Streptomycin and PAS (6 weeks)					No. 469, Patient TL Isoniazid and PAS (10 weeks)					No. 392, Patient VH Isoniazid and PAS (12 weeks)				
	Weeks of Incubation:																			
	3	6	9	16	30	3	6	9	16	30	3	6	9	16	30	3	6	9	16	30
LJ	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	X	X	X	X	X
BA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2c	3c	3c
OAA	0	0	0	4c	4c	0	0	0	0	2c	0	0	0	2c	3c	0	0	0	0	37c
	0	0	0	1c	1c	0	0	0	0	13c	0	0	0	2c	2c	0	0	0	25c	1+
Enriched	0	0	0	3c	3c	0	0	0	1+	1+	0	0	1c	4c	4c	0	0	0	X	X
PABA																				
10	1c	4c	4c	5c	5c	2+	2+	2+	2+	2+	1c	6c	8c	8c	8c	0	1+	1+	1+	1+
25	0	1c	1c	2c	2c	0	X	X	X	X	1c	4c	4c	4c	4c	—	—	—	—	—
100	1c	3c	3c	3c	3c	2+	2+	2+	2+	2+	0	3c	3c	3c	4c	—	—	—	—	—

* 0 = no growth visible; c = number of colonies (1–49); 1+ = 50–100 colonies; 2+ = 100–200 colonies; X = culture contaminated; – = tube not included.

† LJ: Löwenstein-Jensen; BA: blood agar plus penicillin; OAA: oleic-acid albumin plus penicillin; enriched: OAA plus biotin and pyridoxine; PABA: enriched OAA plus indicated concentration of PABA in γ per ml.

TABLE 2

EFFECT OF INCUBATION TIME ON YIELD FROM POSITIVE SPECIMENS

Type of Medium*	Per Cent Positive After Incubation for — Weeks:											
	1	2	3	4	5	6	9	12	16	20	25	30
LJ	0	0	19	27	31	37	44	44	44	44	45	45
BA	0	6	29	44	52	55	60	60	60	60	60	60
OAA	0	4	21	30	36	41	50	59	64	67	67	67
Enriched	0	6	30	42	50	54	68	78	80	82	82	82
PABA†	4	26	54	70	76	80	84	84	86	86	86	86

* See footnotes to table 1.

† Concentration: 10 γ per ml.

PABA. Cultures were examined at weekly intervals for twelve weeks, and at sixteen, twenty, twenty-five, and thirty weeks after inoculation. Of the 83 specimens inoculated, 50 yielded one or more cultures of *M. tuberculosis*.

In table 1 are shown some of the readings of a few selected specimens from different patients which gave evidence of delayed emergence of growth or none on all of the usual diagnostic media. All tubes containing PABA yielded typical colonies from these specimens within four weeks of incubation or less.

The yield of cultures positive for *M. tuberculosis* from all 50 of the specimens after indicated incubation periods may be noted in table 2. Some positive cultures appeared after six weeks' incubation with each type of medium used, but from this series the effect of delayed emergence of positive cultures was most marked with the oleic-acid–albumin medium—an additional 63 per cent of those positive at six weeks had become positive after thirty weeks—and with the enriched medium, which yielded an additional 52 per cent.

Inclusive explanations for delayed emergence of tubercle bacilli must always consider the possibility of contamination. In this study the screw caps on culture tubes were tightened within the first week or two after inoculation and thereafter were kept airtight until macroscopic colonies appeared. In view of the confirmation achieved with duplicate tubes, it is believed that the probability of contamination has been so reduced that it may be safely excluded as a reasonable explanation of results.

* * *

Since the phenomenon of delayed emergence of growth may be the *in vitro* counterpart (and possible cause) of clinical relapse in a treated patient, possible explanations for its occurrence are worth considering. The following hypothesis is advanced as that best able to explain the facts as understood by the author.

Exposure of drug-susceptible tubercle bacilli to even extremely high drug concentrations for a period of weeks has been shown to produce only a reversible bacteriostatic effect when the exposure occurred under specific conditions which did not permit multiplication of the organisms.[12] Exposure of organisms of the same degree of drug susceptibility to similar drug concentrations under conditions permitting multiplication normally would produce irreversible changes resulting in the death of the organisms.

Furthermore, studies[13] with radioactive isotopes have shown that drug-susceptible

[12] FISHER, M. W.: Proc. Soc. Exper. Biol. & Med., 1954, *85*, 538.

[13] BARCLAY, W. R., *et al.*: Am. Rev. Tuberc., 1954, *70*, 784.

tubercle bacilli may adsorb and bind firmly an antituberculous drug when exposed under conditions which do not permit growth of the organisms; drug-resistant organisms did not bind the drug to nearly the same extent. Such adsorbed drug remained too firmly bound to permit removal by ordinary dilution or washing.

The possibility exists that drug-susceptible organisms present in a patient may become exposed to the drug under conditions which would permit adsorption of the drug while the bacilli still remained viable. The organisms could be in a nonmultiplying or latent state because of the lack of essential metabolites or for other reasons, such as biochemical environment or borderline concentration of an inhibitor. Such exposure could conceivably occur within the lungs, in the mouth, or even in the sputum after its collection if portions of orally administered drug should enter the sputum collection jar. Upon removal of such organisms to a growth medium containing all of the usual requirements for growth of tubercle bacilli, the adsorbed inhibitor, if not present in a lethal concentration, could still prevent multiplication of the organisms. This inhibition of growth would last until the death of the organisms, until the inhibitor was chemically changed into a noninhibitor or became detached from the organisms, or until natural antagonists to it present in the medium in small quantities could diffuse in to neutralize its effect, whichever occurred first. All except the first of these conditions would result in delayed emergence of growth of organisms.

If, however, the organisms were transferred to a medium containing adequate quantities of an effective antagonist to the inhibitory drug as well as all other necessary growth requirements, the inhibiting effect could promptly be neutralized, and growth would result after a normal incubation period.

Thus, unless the drug exposure is neutralized by constituents of the medium, one would, in effect, be performing a drug-susceptibility test rather than a culture for isolation under optimal conditions.

If the above conditions, as postulated, do explain the delayed emergence of growth of tubercle bacilli, then accurate tests for viability of tubercle bacilli can only be made in media containing effective antagonists to inhibitory agents. Instead of incubating cultures for prolonged periods (up to a year, as has recently been recommended[3]), increased efforts should be made to define and use growth media containing effective antagonists to inhibitory drugs.

In summary: The incidence of delayed emergence of tubercle bacilli was studied in various media. Portions of specimens from patients receiving PAS produced typical growth after two to four weeks of incubation on media containing added PABA, whereas in many instances the same specimens yielded only delayed growth (appearing after nine to thirty weeks of incubation) or no growth on the same and other media without the PABA. These findings are interpreted to indicate that the delay is due to inadequacies in the growth media rather than to damage done to the organisms.

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CYCLOSERINE ALONE AND IN COMBINATION WITH OTHER DRUGS IN EXPERIMENTAL GUINEA PIG TUBERCULOSIS[1]

Reports from this laboratory[2] and from several others have indicated that cycloserine alone has a low degree of effectiveness in the therapy of tuberculosis in guinea pigs and mice; a somewhat higher degree in rabbits and monkeys (reviewed in reference of footnote 2). Recently, there have been reports of more favorable results when cycloserine was given in combination with isoniazid in monkeys[3] and in man.[4, 5] The results of an experiment in which tuberculous guinea pigs were treated with either cycloserine, pyrazinamide, streptomycin, isoniazid, or combinations of cycloserine with each of the other drugs, are presented in this communication.

Guinea pigs were infected intracardially with approximately five million viable units of *M. tuberculosis* var. *hominis*, strain H37Rv. Treatment was started eight days later according to the following nine regimens: Cycloserine,[6] 135 mg. per kg. per day (100 mg. once daily); cycloserine, 270 mg. per kg. per day (100 mg. twice daily); pyrazinamide,[7] 190 mg. per kg. per day (150 mg. once daily); streptomycin,[8] 27 mg. per kg. per day (20 mg. once daily); isoniazid,[8] 6.7 mg. per kg. per day (5 mg. once daily); cycloserine, 100 mg. plus pyrazinamide, 150 mg.; cycloserine, 100 mg., plus streptomycin, 20 mg.; cycloserine, 100 mg., plus isoniazid, 5 mg.; and cycloserine, 100 mg., plus streptomycin, 20 mg., plus isoniazid, 5 mg.

Isoniazid and streptomycin were given intramuscularly in aqueous solution. Cycloserine and pyrazinamide were administered by mouth in capsules. During the first two days of treatment cycloserine was given intramuscularly in unbuffered aqueous solution. The injections resulted in severe local necrotic reactions which persisted for many weeks.

The treatment of 10 animals in each group was discontinued after sixty-one days, following which these guinea pigs were observed for a further one hundred and fifty days before they were sacrificed. The remainder of the animals in each group were treated for one hundred and fourteen days and then were observed for a further one hundred and seventy-seven days. Tubercle bacilli were recovered from many of the treated guinea pigs by homogenizing the entire spleen in a small amount of physiologic saline with the aid of a mortar and pestle. Aliquots of the homogenate were planted on solid American Trudeau Society medium before and after digestion with 2 per cent sodium hydroxide. *In vitro* drug-susceptibility tests of the recovered strains were performed in the usual manner on inspissated egg media. Susceptibility to pyrazinamide was determined in acidified media as outlined previously.[9]

Comparison of the efficacy of the nine treatment regimens was made by noting the survival time, relapse rate after discontinuance of treatment, the amount of generalized

[1] This study was aided in part by a grant, E 36 (C 10), from the Division of Research Grants and Fellowships, National Institutes of Health, Public Health Service.

[2] Steenken, W., Jr., and Wolinsky, E.: Am. Rev. Tuberc., 1956, *73*, 539.

[3] Schmidt, L. H.: Paper No. 57, Fifteenth Conference on the Chemotherapy of Tuberculosis, Veterans Administration, Army, and Navy, February, 1956.

[4] Epstein, L. G., and Nair, K. G. S.: Paper No. 58, Fifteenth Conference on the Chemotherapy of Tuberculosis, Veterans Administration, Army, and Navy, February, 1956.

[5] Ravina, A., and Pestel, M.: Presse méd., 1956, *64*, 1241.

[6] Generously supplied, as calcium oxamycin, by Merck & Company. Dosage was calculated on the basis of cycloserine base, 135 mg. of the calcium salt being equivalent to 100 mg. of base.

[7] Generously supplied by Merck & Company.

[8] Generously supplied by Chas. Pfizer & Company.

[9] Steenken, W., Jr., *et al.*: Submitted for publication.

TABLE 1

SURVIVAL TIME OF CONTROL AND CYCLOSERINE-TREATED GUINEA PIGS DURING FIRST 69 DAYS AFTER INFECTION (61 DAYS OF TREATMENT)

Treatment	Number of Pigs	Per Cent Mortality	Median Survival Time, Days
Untreated	10	90	53
Cycloserine 135 mg/kg/day	19	79	46
Cycloserine 270 mg/kg/day	20	95	33

TABLE 2

RESULTS OF 61-DAY COURSE OF TREATMENT. SURVIVORS SACRIFICED 150 DAYS POST THERAPY

Treatment Regimen	Number Alive After 61 Days of Treatment	Per Cent Mortality During 150 Days Post Therapy	Survival in Days Post Therapy	Average Degree of TB* in 150-Day Survivors
Pyrazinamide	10	70	2, 2, (32),† (36), (55), 69, 133	8.6
Cycloserine-pyrazinamide	10	100	(8), 9, 37p, 41, 44, 47, 48, (53), 86, 133	—
Streptomycin	10	40	25p, 37, 42p, 134	8.9
Cycloserine-streptomycin	10	90	(11), 28p, (33), 38p, 41p, 52p, 84p, 131p, 136	11.5
Isoniazid	10	20	140, (143)	8.3
Cycloserine-isoniazid	10	0		6.0
Cycloserine-streptomycin-isoniazid	10	0		6.3

* Based on value of 0 to 4 for each of lungs, liver, spleen, and lymph nodes according to amount of gross tuberculous disease. Maximum was 16 for each animal.

† Plain figure indicates a death from generalized tuberculosis; a figure in parentheses indicates a death from unknown cause with little or no generalized tuberculosis; a figure followed by "p" indicates that the animal was paralyzed and presumably died of tuberculous meningitis.

tuberculosis in the animals sacrificed at the end of the observation period, and the number of animals yielding viable tubercle bacilli.

The results of this experiment are presented in tables 1, 2, and 3. From table 1 it may be seen that the administration of cycloserine alone was completely ineffective. In fact, the treated animals died more rapidly than the controls. At autopsy, generalized tuberculosis was seen in all of the control and cycloserine-treated guinea pigs, although some of the early deaths in the treated group were hastened by severe local necrotic reactions produced by the intramuscular injection of cycloserine during the first two days of therapy.

There were no deaths during the first sixty-one days of therapy among the other treated groups. The results presented in tables 2 and 3 indicate that the slight beneficial effect produced by the administration of pyrazinamide alone was not enhanced by the combination cycloserine-pyrazinamide. Similarly, the combination cycloserine-strepto-

TABLE 3

RESULTS OF 114-DAY COURSE OF TREATMENT. SURVIVORS SACRIFICED 177 DAYS POST THERAPY

Treatment Regimen	Died During 61st–114th Day of Treatment		Per Cent Mortality During 177 Days Post Therapy	Survival in Days Post Therapy	Average Degree of TB† in 177-Day Survivors	Number with Positive Spleen Cultures/ Number of Survivors
	Number*	Days of Therapy				
Pyrazinamide	4/9	98, 98, 104, 104	80	12, (45),‡ (80), 113p	11.0	—
Cycloserine-pyrazinamide	3/9	98, 98, 100	50	15, 31, 62	9.2	—
Streptomycin	0/10		60	(35), 78p, 84p, 87p, 89p, 162p	7.4	—
Cycloserine-streptomycin	1/9	(98)	50	(30), (36), 55p, 84p	8.8	—
Isoniazid	0/10		0		5.7	8/10
Cycloserine-isoniazid	0/10		0		3.7	6/10
Cycloserine-streptomycin-isoniazid	0/10		0		5.7	8/10

* Numerator—number that died during therapy; denominator—number still alive on sixty-first day of treatment.

† Based on value of 0 to 4 for each of lungs, liver, spleen, and lymph nodes according to amount of gross tuberculous disease. Maximum was 16 for each animal.

‡ Plain figure indicates a death from generalized tuberculosis; a figure in parentheses indicates a death from unknown cause with little or no generalized tuberculosis; a figure followed by "p" indicates that the animal was paralyzed and presumably died of tuberculous meningitis.

mycin added nothing to the moderately beneficial results afforded by the administration of streptomycin alone. Comparison of the cycloserine-isoniazid and isoniazid alone regimens indicates that the former was slightly superior in regard to mortality after discontinuance of treatment and amount of tuberculous disease in the survivors. At the end of the observation period following the one hundred and fourteen-day course of treatment, viable tubercle bacilli were recovered from the spleens of 8 of 10 animals treated with isoniazid and from 6 of 10 treated with cycloserine-isoniazid. This difference is not considered to be clearly significant. The response of the group of animals treated with a combination of three drugs, cycloserine-streptomycin-isoniazid, was not significantly different from that in those treated with either of the other two regimens containing isoniazid.

It was noted in a previous experiment[5] that guinea pigs infected intracardially with virulent tubercle bacilli often developed tuberculous meningitis which was difficult to eradicate by chemotherapy. As indicated in tables 2 and 3, progressive paralysis presumably due to meningitis was observed in the present experiment in the groups treated with pyrazinamide, streptomycin, and combinations of cycloserine with either of these two drugs, but not in the animals treated with any of the regimens containing isoniazid. The guinea pigs treated with cycloserine alone did not live long enough for meningitis to develop.

Cultures of tubercle bacilli were recovered from some of the treated animals in each

group after various durations of therapy. *In vitro* resistance to pyrazinamide was observed frequently[9] in strains recovered from pyrazinamide-treated guinea pigs, and the addition of cycloserine to the pyrazinamide regimen did not decrease the incidence of pyrazinamide resistance. In addition to cultures obtained from pyrazinamide-treated animals, 36 guinea pig strains were tested for susceptibility to the drugs with which the animals had been treated. With one exception, all of these strains were fully susceptible *in vitro* to streptomycin, isoniazid, or cycloserine. The exception was a strain recovered from an animal killed one hundred and fifty days following sixty-one days of cycloserine-isoniazid administration; this strain was completely susceptible to cycloserine but highly resistant to isoniazid, and catalase positive.

In summary: Combination therapy with cycloserine-streptomycin and cycloserine-pyrazinamide produced results similar to those observed with streptomycin or pyrazinamide alone in guinea pigs infected via the blood stream with *M. tuberculosis*. Beneficial effects derived from the administration of cycloserine-isoniazid were slightly superior to those from isoniazid alone, but the difference between the two regimens was not striking, and the over-all effectiveness of the cycloserine-isoniazid regimen was inferior to that observed with pyrazinamide-isoniazid in previous experiments[9] from this laboratory. Cycloserine alone, in a dosage of 270 mg. per kg. per day, was ineffective. *In vitro* resistance to cycloserine was not observed in any of the cultures isolated from cycloserine-treated guinea pigs. The administration of cycloserine with pyrazinamide did not influence the emergence of tubercle bacilli resistant to pyrazinamide.

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SOME CONSIDERATIONS OF CYCLOSERINE TOXICITY

Clinical experience with cycloserine for eighteen months has demonstrated not only a very definite convulsive toxicity, but also the particular circumstances under which such convulsions occur.

Cycloserine was found to be a drug of relatively low toxicity in laboratory animals.[1] Acute toxicity studies showed that the lethal doses were extremely high and that it was not practical to determine them in large animal species. Chronic toxicity studies, using continuous medication with high doses, resulted in no abnormalities in rats, cats, dogs, or monkeys.

In Schmidt's[2] studies of the effect of cycloserine alone in the treatment of simian tuberculosis, using the rhesus monkey as his test object, he employed daily doses of 400 mg. per kg., 200 mg. per kg., and 100 mg. per kg. In the group which received 100 mg., there was no significant toxic effect. In contrast, the 400 mg. dose schedule had to be abandoned because of its severe neurotoxic effect, consisting for the most part of marked sedation, but also, with some convulsions. The 200 mg. group usually showed a transient, sedative effect.

In clinical use the administration of cycloserine alone to humans has at times been attended by symptoms of neurotoxicity, such as drowsiness, somnolence, hyperreflexia, mental confusion, and convulsions. The occurrence of convulsions has been striking in its frequency.

Of the original group of 29 patients who were treated with cycloserine alone, 6 patients suffered generalized clonic convulsive seizures.[3] Certain striking similarities among these patients attracted attention and are shown in table 1.

If *Case 6*[4] may be disregarded for purposes of discussion, since the patient did not fit the pattern of the group, the other 5 patients showed the following features in common.

> They were all receiving 1.0 gm., or more, of cycloserine per day. The drug was given in equally divided doses at 7 a.m. and 7 p.m. so that each patient was taking individual doses of 0.5 gm. (4 cases) or 0.75 gm. (one case).
>
> All of the convulsions occurred at approximately the noon hour, between 11:20 a.m. and 12 N. This was four and one-half to five hours after the morning dose of cycloserine, which is approximately the time of the expected peak of drug concentration in the blood.
>
> All five convulsions occurred within thirty-three days after the start of treatment with cycloserine.
>
> Four of these patients were subsequently able to resume cycloserine therapy at a reduced dose without further difficulty.

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[1] Anderson, R. C., *et al.*: Antibiot. & Chemother., 1956, *6*, 360.

[2] Schmidt, L. H.: Personal communication to Dr. R. L. McLean.

[3] Storey, P. B., *et al.*: Antibiot. Med., 1956, *3*, 258.

[4] Although the patient (*Case 6*) differed from the others in that a convulsion did not occur until the seventh month of treatment, toxic symptoms (headache, malaise, nervousness, and twitching of the left arm) began eight days after the dose of cycloserine had been increased to 1.0 gm. every twelve hours. Cycloserine was discontinued on that day. In spite of discontinuance of the drug and improvement of the patient's symptoms, he had a severe clonic convulsion twenty-eight hours after his last dose of cycloserine, following which his blood concentration of cycloserine was found to be 26.5 γ per ml.

TABLE 1

CONVULSIVE EFFECT OF CYCLOSERINE

Case	Daily Dose of Cycloserine	Time of Day Convulsion Occurred	Interval Since Last Dose	Cycloserine Blood Concentrations at Time of Convulsion	Comments
				γ/ml.	
1	1 gm., 0.5 gm. every 12 hours	12: N	5 hours	27.5	
2	1 gm., 0.5 gm. every 12 hours	12: N	5 hours	26.0	Resumed cycloserine therapy at 1.0 and 1.5 gm. per day
3	1.5 gm., 0.75 gm. every 12 hours	11:20 a.m.	4½ hours	35.0	Resumed cycloserine therapy at 0.5 gm. per day
4	1 gm., 0.5 gm. every 12 hours	12: N	5 hours	35.5	Resumed cycloserine therapy at 0.75 gm. per day
5	1 gm., 0.5 gm. every 12 hours	11:25 a.m.	4½ hours	36.0	Resumed cycloserine therapy at 0.5 gm. per day
6	2 gm., 1.0 gm. every 12 hours	12:35 p.m.	29½ hours	26.5	

TABLE 2

USPHS INVESTIGATION OF CYCLOSERINE TOXICITY: FRANCIS J. MURRAY[5]

	Number of Patients Treated		Toxic Reactions	Convulsions
TOTAL		115	18	8
1 gm. per day		25		
0.5 gm. twice daily	25		11 (44%)	4
0.5 gm. per day		90		
1.0 gm. every second day	39		5 (12.8%)	3
0.5 gm. once daily	38		2 (5.3%)	1
0.25 gm. twice daily	13		—	—

These similarities are striking but assume their full significance in the light of two other published studies of cycloserine effect.

The incidence of convulsive toxicity in the writers' group of patients was at marked variance with that reported by Epstein, Nair, and Boyd.[5] They reported only two instances of convulsive effect in fifty-seven patients treated with 1.0 to 1.5 gm. of cycloserine per day, one of these being a known epileptic under control with Dilantin® therapy. It is important to emphasize here that, whereas in the present series the drug was administered every twelve hours in two equally divided doses, Epstein, Nair, and Boyd administered the cycloserine in four equally divided doses.

The United States Public Health Service cooperative study of the toxicity of cycloserine, reported by Murray, indicates a convulsive toxicity of similar incidence to that in the present series, and appears to explain the discrepancy between the writers' findings and those of Epstein.[6] Table 2 is constructed from Murray's report with his permission.

[5] EPSTEIN, I. G., *et al.*: Dis. of Chest, 1956, *29*, 241.

[6] MURRAY, F. J.: Am. Rev. Tubere., 1956, *74*, 196.

It may be seen from this table that 4 of 25 patients who received 1.0 gm. per day of cycloserine (0.5 gm. twice daily) suffered convulsions. Even more interesting, however, is the group of 90 patients who were given an average dose of 0.5 gm. per day according to three different schedules. Of the 39 patients who received 1.0 gm. in a single dose every second day, 3 had convulsions. Of the 38 patients who were given 0.5 gm. once daily, one had a convulsion. And in the group of 13 patients who received 0.25 gm. twice daily there were no toxic effects.

* * *

These three clinical studies indicate that cycloserine acts as a simple epileptisant, and that this effect is related to both the total daily dose and to the size of the individual dose. It would certainly seem from these studies that the individual dose should not exceed 0.25 gm., regardless of the total daily dose.

It is probably appropriate to point out here that there is still no more detailed knowledge of the metabolism of cycloserine in the body than that reported by Conzelman in his studies with rhesus monkeys.[7] He pointed out that only 43 per cent of an intramuscular dose, and 25 per cent less of an oral dose, was excreted in the urine. He sought to emphasize that the major fraction of cycloserine, which does not appear in the urine after either intramuscular or oral administration, must be metabolized to a substance or substances not yet defined. The relationship between the intermediary metabolism of cycloserine and its neurotoxicity remains, therefore, undisclosed.

In summary: The following four conclusions should serve as guides in the clinical use of cycloserine:

Cycloserine has a definite neurotoxic effect, which may be sedative or excitatory, but which in its most dramatic form is convulsive.

The convulsive effect of cycloserine appears to be directly related to the total daily dose of the drug used and to the size of the individual dose administered.

The convulsive effect of cycloserine is greatly diminished, and may be abolished, by not exceeding a total daily dose of 0.5 gm. or an individual dose of 0.25 gm.

If a daily dose of 1 gm. is used, it should not be given in equally divided 0.5 gm. doses, but should be given in 0.25 gm. divided doses.

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MODE OF ACTION OF ISONIAZID

To the Editor of The American Review of Tuberculosis and Pulmonary Diseases:

Current interest in the mode of action of isoniazid is reflected by the wealth of recent literature on the subject. Middlebrook[1] noted that strains of *M. tuberculosis* which are highly resistant to isoniazid and nonpathogenic for guinea pigs were consistently catalase negative. Pope[2, 3] noted that the inhibition of endogenous respiration of *M. tuberculosis* could be completely neutralized by the addition of pyridoxal or alpha-ketoglutarate but not by pyruvate. Barclay and associates[4, 5] reported that the fixation of isoniazid to susceptible tubercle bacilli is governed by a mechanism resembling simple physical adsorption. Pätiälä[6] noted a fall in pyridine nucleotide concentration in the blood of experimentally infected tuberculous animals which returned to normal on administration of isoniazid, and concluded that isoniazid acts by inhibiting enzyme reactions in which pyridine nucleotides are involved. Goldman[7] isolated the isoniazid analogue of diphosphopyridine nucleotide and observed that it was not a suitable electron acceptor for many pyridine nucleotides requiring oxidative enzymes. He also noted that the analogue had no inhibitory effect on the reduction of diphosphopyridine nucleotide or triphosphopyridine nucleotide by their specific dehydrogenases. He concludes that the antituberculous activity of isoniazid may therefore be due to the intracellular formation of an inactive analogue with a concomitant reduction in cellular oxidative metabolism. Lichstein[8] suggested that isoniazid exerts its primary competitive effect on the vitamin B_6 group by competing with pyridoxal phosphate for the apoenzyme. Neumayr and associates[9] and later Knox and associates[10] confirmed and expanded Middlebrook's[1] earlier observations on the relationship of catalase activity to pathogenicity and isoniazid susceptibility. Carl and Marquardt[11] suggested a direct quantitative relationship between the antituberculous activity of *p*-aminosalicylic acid, isoniazid, and certain benzal thiosemicarbazones and their ability to form complexes with cupric ion. Later Foye and Jeffrey[12] found that this relationship was valid as well for a group of *o*-hydroxyazonapthols and phenanthrols. However, Foye[13] observed a decrease of antituberculous activity when *p*-aminosalicylic acid was complexed with copper. Cymerman-Craig and associates[14] confirmed the earlier observations[11] on the copper chelate of isoniazid and pointed out the structural resemblance it bore to a metal chelate of pyridoxal and an amino acid. Rubbo

[1] Middlebrook, G.: Am. Rev. Tuberc., 1954, *69*, 471.
[2] Pope, H.: Am. Rev. Tuberc., 1953, *68*, 938.
[3] Pope, H.: Am. Rev. Tuberc., 1956, *73*, 735.
[4] Barclay, W. R. *et al.*: Am. Rev. Tuberc., 1953, *67*, 490.
[5] Barclay, W. R. *et al.*: Am. Rev. Tuberc., 1954, *70*, 784.
[6] Pätiälä, J.: Am. Rev. Tuberc., 1954, *70*, 453.
[7] Goldman, D.: J. Am. Chem. Soc., 1954, *76*, 2841.
[8] Lichstein, H. C.: Proc. Soc. Exper. Biol. & Med., 1955, *88*, 519.
[9] Neumayr, R. B., *et al.*: Proc. Soc. Exper. Biol. & Med., 1955, *89*, 468.
[10] Knox, R., *et al.*: Am. Rev. Tuberc., 1956, *73*, 726.
[11] Carl, E., and Marquardt, P. Z.: Naturforsch., 1949, *4*, 280; 1952, *7*, 575.
[12] Foye, W. O., and Jeffrey, J. G.: J. Am. Pharm. A., 1955, *44*, 257.
[13] Foye, W. O.: J. Am. Pharm. A., 1955, *44*, 415.
[14] Cymerman-Craig, I., *et al.*: Nature, 1955, *176*, 34.

TABLE I

Catalase Preparation	Per Cent of Catalase Activity			
	Control (No Additions)	Cu^{++} Added	Isoniazid Added	Cu^{++} and Isoniazid Added
Bovine liver	100	59.0	78	12.0
BCG, whole cell suspension	100	82.5	78	27.0
BCG, cell-free extract	100	86.0	82	42.5

* Cu = copper.

and co-workers[15] showed that the chelation of isoniazid with a metal is a necessary prerequisite for its antituberculous activity both *in vitro* and *in vivo*.

From this brief review three facts concerning the mode of action of isoniazid seem to be fairly well established: (*1*) There is a direct relationship between isoniazid susceptibility, pathogenicity, and catalase activity. (*2*) Isoniazid must form a metal chelate *in vitro* and *in vivo* before its antituberculous potential can be realized. (*3*) Isoniazid metal complexes somehow interfere with oxidative enzyme reactions involving pyridine nucleotides.

About a year ago this laboratory embarked upon an investigation of the role of metal chelation in the kinetics of inhibition of catalase by isoniazid. Preliminary studies showed a remarkable increase in inhibition of catalase from bovine liver and from BCG when the isoniazid was complexed with copper. Cell-free extracts with intact catalase activity were then prepared by sonic dispersion from multiplying BCG cultures, and the kinetics of isoniazid and isoniazid copper complex inhibition of this system were studied. As in the case of the particulate BCG catalase in intact cells, the inhibition kinetics observed were those of a weakly competitive system in the absence of copper and a strongly competitive system in the presence of copper. When a random group of ten additional metals was studied, using bovine liver catalase in the same manner, their behavior paralleled that of copper with one exception, silver. They differed from copper only in the degree of additional inhibition which they conferred on isoniazid.

Based on these preliminary observations, an exhaustive investigation of this phenomenon was planned and is presently in execution. Cell-free extracts of a series of clinically interesting strains of *M. tuberculosis* which have been extensively investigated bacteriologically are being prepared for a kinetic study of the inhibition of their catalase by isoniazid and certain isoniazid analogues in the presence and absence of exogenous metals. The preliminary observations described above and summarized in the accompanying table suggest the possibility that isoniazid's mode of action may simply involve formation of a metal chelate which then competes with hydrogen peroxide for possession of sites on the catalase molecule. This would result in an accumulation of hydrogen peroxide and the death of the bacilli.

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August 23, 1956

[15] Rubbo, S. D., *et al.*: Nature, 1956, *177*, 4506.

VIABLE AND STAINABLE COUNTS ON TUBERCLE BACILLI IN TUBERCULOUS TISSUE

To the Editor of The American Review of Tuberculosis and Pulmonary Diseases:

There are many references describing the inefficiency of microscopic examination for tubercle bacilli in sputum and tuberculous tissues.[1-6] That the insensitivity of this method is due to purely mathematical factors has been clearly explained.[7, 8] It has also been established[9-11, 13] that viable organisms can be demonstrated in tuberculous tissue by cultural and animal infectivity tests even though no visible acid-fast bacilli can be detected. This apparent difference between stainable and viable counts might be attributed to granular and other forms of tubercle bacilli in tuberculous tissue which are not detectable by staining.[11] However, it has been shown by Fenner[12] that with careful examination of culture suspensions of tubercle bacilli the number of stainable organisms is not significantly different from the viable count. Using essentially the same technique as that described by Fenner, we have investigated the apparent discrepancy between viable and stainable bacilli in tuberculous tissue, using infected mouse lung as the source of our material. This work was stimulated by the recent observations of Gray and Jennings[13] who, like other observers, had noted the discrepancy referred to above.

C57 mice were infected intranasally with less than 1,000 viable units of the H37Rv strain of *M. tuberculosis*. At various times after infection, mice were killed, the lungs were removed aseptically and homogenized with Ringer's saline in a Waring Blendor, and decimal dilutions were made in 0.1 per cent bovine albumin V in distilled water. Counts were made by dropping pipette (Pasteur pipette calibrated so that one drop is approximately equal to 0.02 ml.) onto the surface of Löwenstein's medium and on to cleaned microscopic slides. In order that dilution and mixing errors would not influence the comparison of the two counts, drops for both counts were put out from the same pipetteful of suspension. The drops for the counts of stainable organisms were allowed to dry at room temperature, were stained with auramine-rhodamine and examined by fluorescence microscopy.[6, 13, 14] The stained preparation was counted in such a way that the whole of the drop-smear was examined. Counts on replicate drops were so remarkably uniform that, though at first 10 to 12 drops were counted from each suspension, later only 5 drops were counted. Viable counts were made by inoculating three drops of each dilution onto each of three tubes of Löwenstein's medium and incubating for twenty-five days at 37° C. In this work it has been found that the maximal viable count is obtained with this incubation time, since

[1] Smith, C. R.: Am. J. Clin. Path., 1951, *21*, 674.
[2] Widelock, D., *et al.*: Am. Rev. Tuberc., 1954, *70*, 349.
[3] Berry, J. W., and Lowry, H.: Am. Rev. Tuberc., 1949, *60*, 51.
[4] Cummings, M. M.: Am. J. Pub. Health, 1949, *39*, 361.
[5] Cummings, M. M.: Am. J. Clin. Path., 1951, *21*, 684.
[6] Gray, D. F.: Am. Rev. Tuberc., 1953, *68*, 82.
[7] Corper, H. J.: J.A.M.A., 1928, *91*, 371.
[8] Editorial, J.A.M.A., 1954, *154*, 1424.
[9] Brieger, E. M., *et al.*: J. Hyg. (Camb.), 1951, *49*, 189.
[10] Nouffland, H., and Berteaux, S.: Am. Rev. Tuberc., 1955, *72*, 330.
[11] Rich, A. R.: The Pathogenesis of Tuberculosis, Charles C Thomas, Springfield, Illinois, 1951.
[12] Fenner, F.: Am. Rev. Tuberc., 1951, *64*, 353.
[13] Gray, D. F., and Jennings, P. A.: Am. Rev. Tuberc., 1955, *72*, 171.
[14] Wilson, M. M.: Am. Rev. Tuberc., 1952, *65*, 709.

further incubation only reduces the count through coalescence of colonies. In all, fifteen suspensions of lung tissue covering all stages of infection from seven to one hundred and twenty days were examined and the following results are typical for the series.

Condition of Mouse Duration of Infection Footpad Reaction*	Logarithm of Number of Organisms per Lung		Ratio of Stainable/Viable Count
	Staining	Cultural	
Healthy, 7 days, −ve (pre-allergic)	5.66	5.06	1.12
Healthy, 28 days, +ve	7.37	7.09	1.04
Healthy, 42 days, +ve	7.15	6.70	1.07
Moribund, 21 days, −ve (pre-allergic)	9.65	9.28	1.05
Moribund, 120 days, −ve (terminal anergic)	7.83	7.60	1.03

* Tuberculin, 0.03 ml. of 1:25 dilution into footpad.[13]

As may be seen, the stainable counts are at least equal to the corresponding viable counts. These results do not support the view that the stainable count of acid-fast organisms in tuberculous tissue is significantly lower than the viable count. However, we would agree that, unless meticulous care is given to the enumeration of stainable organisms, this count will be invariably lower than the viable count. The difference is apparently a measure of the inefficiency of the staining method and it is not necessary to invoke the presence of viable, submicroscopic or of nonacid-fast elements to explain it.

In this connection we have noted that the morphology of organisms from tuberculin-negative mice or from those which have recently "converted" closely resembled in size and shape the organisms seen in dispersed fluid cultures, whereas organisms in tissues of mice which had been tuberculin positive for some time (approximately thirty days) were distinctly slender. This slenderness renders the organisms much more difficult to visualize and may, in part, account for the difficulty in demonstrating acid-fast bacilli in tissue sections. A constant feature of lung tissue suspensions in this study was the complete dispersal of the bacilli. Mouse lung has interesting possibilities as a source of single bacillary cells, especially in the pre-allergic stage of infection.

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December 14, 1956

Books

Socio-Economic Conditions and Tuberculosis Prevalence, New York City, 1949–1951. By Anthony M. Lowell. New York Tuberculosis and Health Association, New York, N. Y., 1956.

This volume reports a joint effort by the New York Tuberculosis and Health Association and the National Tuberculosis Association. It correlates the facts of active tuberculosis prevalence rates in the years 1949 to 1951 with socio-economic conditions revealed in New York City by the 1950 census and other studies.

The tuberculosis prevalence rate is defined as the ratio per 100,000 population of cases known on the first day of the year plus new cases reported during the year plus former cases becoming active, exclusive of those diagnosed inactive or arrested. An average value for the three years, 1949–1951, is used.

For New York City, as for possibly no place else in the world, there is a tremendous amount of data on the health status of the population, status of housing, and various socio-economic indices. The city is divided into thirty health districts which in turn are subdivided into health areas. Because of this, social, economic, and epidemiologic variables can be pin-pointed and correlated to an extent impossible under ordinary circumstances.

The study gives meticulous comparisons through tables and charts of the relationship between the annual prevalence rates, index of housing units (unsanitary and dilapidated), overcrowding, income per family, employment, and other factors.

Significant correlations are demonstrated between tuberculosis and poor housing, between tuberculosis prevalence and racial composition of the population, and between tuberculosis and low income. Charts show prevalence rates by age, sex, and race, which accentuate the need to do better tuberculosis case finding in low income groups, the Negro population, Puerto Rican population, and among the aging.

This is a valuable source book for facts about New York City's tuberculosis problems. More than that, it is of value for objective evidence of the relationship between tuberculosis and the conditions under which man lives. As for methodology, it offers valuable hints to those interested in more accurate knowledge of their own communities, although, perhaps, on a smaller, less extensive scale than that possible in New York City.

As with most studies based upon census year statistics, it must be clearly understood that delays inherent in the system render it impossible to know "current data." This study constitutes a valuable baseline. Perhaps data based upon the 1960 census will enlighten us concerning the effects of the wide use of antimicrobials upon the health problems posed by tuberculosis.

Howard M. Payne
Washington, D. C.

Dermatology. By Donald M. Pillsbury, Walter B. Shelley, and Albert M. Klingman. Pp. xx and 1331, with 564 illustrations, W. B. Saunders Company, Philadelphia, 1956. Price, $20.00.

In this book the reader is prepared for the details of the text by an unusually careful exposition of basic principles. The authors contend that practicing physicians reasonably familiar with the applied anatomy, physiology, and chemistry of the skin, and a dozen

or so of the chief patterns of dermal disease will be able to classify 90 per cent of the skin lesions brought to their attention. The organization of the volume is relatively simple, and the text is obviously based on an extraordinarily wide personal experience. Readers of THE AMERICAN REVIEW OF TUBERCULOSIS AND PULMONARY DISEASES will be especially interested in the sections devoted to allergies of infection, tuberculosis, and the several mycotic granulomas with both pulmonary and dermal manifestations. They will find these sections modern in outlook and practical. It is worthy of note that, although chemotherapy is indicated as the first recourse in the treatment of tuberculosis of the skin, preceding surgical and other physical measures, dosages and regimens of therapy are not nearly so well worked out as they are in pulmonary and other forms of tuberculosis. Illustrations, which are in black and white, are numerous and informative. The press work is excellent.

ESMOND R. LONG,
Pedlar Mills, Virginia

DRIED BCG VACCINE. By Yoji Obayashi. Pp. 214, The World Health Organization, Palais Des Nations, Geneva, Switzerland, 1955. Price, $5.00.

Three of the important problems encountered in mass vaccination are viability, potency, and uniformity of the BCG vaccine. The application of the lyophilization process has been considered a solution to these problems. In Japan, since 1949 considerable work has been done in this connection.

This monograph is based on the findings of the staff of the Research Institute of the Japan Anti-Tuberculosis Association in their attempt to prepare suitable lyophilized BCG for use in vaccination in Japan. Viable plate counts and human inoculation tests were conducted to determine the effects of culture period, method of shaking, culture medium, method of freeze-drying, storage, temperature, light, degree of vacuum, and residual moisture.

Based on these studies a method of production was developed and modified. The present method uses second or third generation nine- to ten-day-old cultures from Sauton's medium. The pellicles are harvested, squeezed dry, weighed (average 20 gm.) and shaken with crystal balls (3,500) for five minutes. Then 5.0 ml. of saccharose (1.25 per cent solution) is added and shaking is continued for one minute. The solution is diluted with more saccharose to a concentration of 10 mg. cells per ml. and is dispensed into ampoules, each containing 0.5 ml. Ampoules are cooled to approximately −20°C. and frozen. Vacuum is applied to a pressure of 0.01–0.001 mg. of mercury for seven hours. After the first hour of vacuum the temperature is raised to 20°C. for approximately one hour. Following lyophilization the ampoules are sealed under vacuum either by hand or mechanically.

Using BCG prepared in this way, the author reports extensive tests which indicate that the viability of the BCG is decreased only slightly by lyophilization. In practice, this can be compensated for safely by the administration of slightly more than the usual 0.1 ml. of vaccine. Viability is relatively stable for one year when the vaccine is stored in a refrigerator. Potency appears to be excellent as the tuberculin conversion rate following intracutaneous administration of the vaccine is usually more than 90 per cent. Elevated temperature (30°–37°C.), light, and storage for more than one year are reported to reduce slightly both viability and potency. The author indicates that further studies on these problems are being conducted in an attempt to improve the product.

It should be noted that throughout the monograph the development of tuberculin hypersensitivity is used as the index of "potency."

This monograph should be of interest to all concerned with the problems of BCG vaccination but is of particular value to those interested in the production of BCG.

JOHN L. SEVER
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BOOKS RECEIVED

IMMUNITY. Hypersensitivity Serology. By Sidney Raffel. Pp. 531, Appleton-Century-Crofts, New York, 1953. Price, $8.00.

HEART DISEASE. By Paul Dudley White. Pp. 1015, Macmillan Company, New York, 1951. Price, $12.00.

CLINICAL ELECTROCARDIOGRAPHY. Part 1. The Arrhythmias. By Louis N. Katz, and Alfred Pick. Pp. 737, Lea and Febiger, Philadelphia, 1956. Price, $17.50.

PAPER ELECTROPHORESIS. Ciba Foundation Symposium. Edited by G. E. W. Wolstenholme and Elaine C. P. Millar. Pp. 224, Little, Brown and Company, Boston, 1956. Price, $6.75.

BONE STRUCTURE AND METABOLISM. Ciba Foundation Symposium. Edited by G. E. W. Wolstenholme and Cecilia M. O'Connor. Pp. 299, Little, Brown and Company, Boston, 1956. Price, $8.00.

PERSONALITY, STRESS AND TUBERCULOSIS. Edited by Phineas J. Sparer. Pp. 629, International Universities Press, New York, 1956. Price, $12.50.

THE MANAGEMENT OF TUBERCULOSIS. Symposium. Edited by Irving J. Selikoff. Pp. 646, Waverly Press, Inc., Baltimore, 1956.

PRINCIPLES OF CHEST X-RAY DIAGNOSIS. By George Simon. Pp. 174. F. A. Davis Company, Philadelphia, 1956. Price, $8.50.

THE MERCK MANUAL OF DIAGNOSIS AND THERAPY, Ed. 9. Edited by Charles E. Lyght. Pp. 1870, Merck and Company, Rahway, N. J., 1956. Price, $6.75.

TUBERKULULOME DER LUNGEN. Mario Alves de Sousa. Pp. 92, Benno Schwabe and Company, Basel, Switzerland, 1956. Price, Fr. 14.

MEDIZINISCHE RONTGENTECHNIK. By Herbert Schoen. Pp. 437, Georg Thieme Verlag, Stuttgart, 1956. Price, $7.00 (Intercontinental Medical Book Corporation, New York, United States representative).

ANATOMIE MEDICO-CHIRURGICALE DU POUMON. By Michel Latarjet and Felix Magnin. Pp. 326 with 155 figures, G. Doin & Cie., Paris-VI^{e}, France, 1956. Price, Fr. 8.500.

GUIDE TECHNIQUE ET TOPOGRAPHIQUE D'EXPLORATION BRONCHOLOGIQUE (Bronchoscopie et Bronchographie). By Jean Ioannou in collaboration with L. Duchet-Suchaux and A. Pinelli. Pp. 113, Masson & Cie., Paris (VI^{e}), France, 1956.

YOUR WORLD AND MINE. By Halbert L. Dunn. Pp. 94, Exposition Press Inc., New York, N. Y., 1956. Price, $3.00.

American Trudeau Society

Medical Section of the National Tuberculosis Association

Female Genital Tract Tuberculosis

A Statement of the Committee on Therapy

Tuberculosis of the female genital tract, symptomatic or asymptomatic, is a serious disease and requires vigorous treatment. Two recently published series emphasize the need for adequate therapy. In the period 1945 to 1947, 28 cases of gynecologic tuberculosis were diagnosed at Cook County Hospital and, by 1949, 18 of the patients were deceased (1). Unfortunately, this report does not give the severity of the pelvic disease or the incidence of associated extra-pelvic tuberculosis. In a series of 109 cases reported from England (2), an attempt was made to use 52 cases as untreated controls for an evaluation of the effects of chemotherapy in the other 57 cases. In 10 of the patients in the control group, the disease deteriorated so badly that chemotherapy had to be instituted. Of the remaining control patients, 25 were re-evaluated at the end of one year and 20 still had active disease. Such reports serve to emphasize the need for early diagnosis followed by appropriate chemotherapy.

The European literature attributes approximately 5 per cent of pelvic inflammatory disease and 5 per cent of sterility problems to pelvic tuberculosis. This figure of 5 per cent is remarkably constant from country to country and from writer to writer. In the United States, however, the incidence is apparently lower than 5 per cent. Schaefer (3) reports that only 0.22 per cent of all patients discharged from the New York Lying-In Hospital between 1951 and 1954 were diagnosed as having pelvic tuberculosis. Brown (4) reports from Baltimore that tuberculosis accounted for 3.9 per cent of chronic salpingitis diagnosed in the period 1920 to 1950.

Genital tract tuberculosis is usually secondary to a primary lesion in the lung. Barnes (5) believes that a hematogenous spread to the pelvic organs is more likely to occur when the primary infection coincides with the period of adolescence or early maturity. Despite the fact that genital tract infection results from a hematogenous spread of bacilli, only a small percentage of these patients have active tuberculosis manifest in other organs (5). A careful search for both active and healed extra-pelvic lesions should be carried out. The fallopian tubes are the pelvic structures most commonly involved (6, 7). From the tubes the infection spreads by direct extension to the endometrium in about 50 per cent of cases. The ovaries are involved in 20 to 30 per cent; the peritoneum, in 50 to 80 per cent of cases. The tubes and ovaries are usually involved bilaterally.

The most common symptom of pelvic tuberculosis is pelvic pain (6), experienced by 50 to 75 per cent of patients. The pain is not usually severe, tends to be rather constant in character, and is frequently accompanied by the complaint of "abdominal swelling." Other complaints are dysmenorrhea, menorrhagia, fatigue, and low-grade fever. However, more than 40 per cent of patients present with only the complaint of infertility. Signs of importance are those of tubo-ovarian masses, peritonitis, or of chronic ulcerations or sinus tracts of the external genitalia. Pelvic inflammatory disease in a virgin or chronic pelvic inflammatory disease that does not respond to antimicrobial treatment is highly suggestive of tuberculous infection. Abnormalities seen on hysterosalpingography (8) may also suggest tuberculosis.

A first and most important step in the diagnosis is tuberculin skin testing. The possibility of the presence of active tuberculosis when a properly performed tuberculin test is negative is very remote. An intracutaneous injection of 0.0001 mg. of PPD is the test procedure recommended. Whereas a negative Mantoux test will almost surely rule out tuberculosis as the cause of the pelvic disease, a positive test will not firmly establish the diagnosis.

The diagnosis should be confirmed when possible by either the demonstration of typical tuberculous lesions in tissue sections or the isolation of tubercle bacilli by culture. It should be emphasized that surgical specimens may require careful serial sectioning to reveal histologic evidence of tuberculosis. Material for culture and histologic sections may be obtained by endometrial biopsy or curettage. Because the endometrium is periodically shed, the demonstration of tubercles or of tubercle bacilli in this tissue signifies active disease. Since the endometrium is involved in only about half of the cases of genital tract tuberculosis, a culture negative for tubercle bacilli does not necessarily signify freedom from tuberculous infection. A negative result may also occur because of an inadequate sample of tissue. In this respect endometrial curettage is superior to a small biopsy. Schaefer (3) suggests that, when a strong possibility of tuberculosis exists, a biopsy every month and a curettage every three months be performed until a diagnosis is established. Biopsy or curettage should be done during the immediate premenstrual phase of the cycle (1). Culturing menstrual blood has also been advocated as a means of diagnosing tuberculous infection (11, 12).

Once the diagnosis has been established, it is helpful to classify the disease into minimal and advanced cases (13). Minimal cases are practically symptomless, there are no palpatory changes in the adnexae, and tuberculosis is discovered in the endometrium by bacteriologic or microscopic examination. Such cases are usually diagnosed in sterility clinics. Advanced cases are usually symptomatic and there are palpable masses in the adnexae.

All patients with active disease should have preliminary periods of hospitalization for observation, completion of diagnostic studies, and the institution of chemotherapy. The duration of hospitalization varies within wide limits. The same principles, including chemotherapy, apply to the treatment of genital tract tuberculosis as apply to the treatment of tuberculosis elsewhere in the body. The kind and the length of bed-rest treatment recommended for any individual patient will depend upon the severity of the pelvic disease and the presence or absence of active extra-pelvic disease. More emphasis should be placed on bed-rest treatment for the young female whose history suggests that the pelvic tuberculosis is the result of a recent postprimary spread than for older patients whose pelvic disease has been present for a long period of time.

When pelvic tuberculosis is first diagnosed at the time of a laparotomy, or following the examination of organs removed surgically, the patient should be treated by a period of bed rest and chemotherapy. Chemotherapy should be given for at least twelve months, but it should be continued even longer if the pelvic tuberculosis discovered is extensive or if extra-pelvic tuberculosis is also subsequently diagnosed.

The drug regimens and duration of treatment that have become accepted as satisfactory for pulmonary and for renal disease should be employed in the treatment of genital tract infection. The following drug regimens are considered to be satisfactory (14):

1. Streptomycin-PAS: 1.0 gm. of streptomycin intramuscularly two or three times weekly and 12 gm. of PAS (or 15 gm. of sodium PAS) daily by mouth in three or four divided doses
2. Isoniazid alone: 5 mg. or more of isoniazid per kg. of body weight per day in two or three divided doses

3. Isoniazid-streptomycin (both daily): 5 mg. or more of isoniazid per kg. and 1.0 gm. of streptomycin intramuscularly
4. Isoniazid daily—streptomycin twice weekly: Same dosage as in *Regimen 3*, but streptomycin given twice weekly instead of daily
5. Isoniazid-PAS: 5 mg. or more of isoniazid per kg. per day and 12 gm. of PAS daily
6. Isoniazid-streptomycin-PAS: each in dosage as above, streptomycin given daily or twice weekly.

Regimens 1 and *2*, streptomycin-PAS and isoniazid alone, appear to be somewhat less effective than the other regimens, as judged by organized cooperative trials in pulmonary tuberculosis. Percentage advantages in favor of *Regimens 3* to *6*, in terms of such clinical criteria as roentgenographic improvement and sputum culture "conversion," are consistently found in various reports of large-scale comparative regimen trials. These advantages, however, are not large and other factors may be considered in selecting a regimen for a given patient with pulmonary and/or extrapulmonary tuberculosis. Regardless of the regimen selected, therapy should be continuous and for a minimal period of twelve months or longer.

The place of surgery in the treatment of genital tract tuberculosis is not as well defined as it was a few years ago. Stallworthy (15) has advocated treating the tuberculous uterus as one would a carcinoma, by complete hysterectomy and salpingo-oophorectomy. Aware that presently available chemotherapy is not completely eradicative, recognizing the potential danger of residual necrotic foci, and realizing that fertility is very seldom restored, there is still reason to adopt a more conservative approach than radical surgery in all cases. Occasionally, conception may follow a period of chemotherapy (16–18) and there is encouraging evidence in the fields of pulmonary and renal tuberculosis that long-term chemotherapy can suppress reactivation of residual infection. The need for surgical treatment can best be determined by treating the patient with a drug regimen and performing a pelvic examination every month to assess the response of adnexal masses to chemotherapy. Those patients originally classified as having minimal disease and those responding by marked regression of the adnexal masses may be continued on chemotherapy alone. Patients who have not shown a marked diminution in adnexal masses after an initial three- to six-month period should be considered for surgery. The extent of the surgery should be governed by the pathologic findings, although a total hysterectomy and bilateral salpingo-oophorectomy is often necessary. Surgery should be followed by chemotherapy for twelve months or longer.

The effectiveness of chemotherapy can be judged by the disappearance of symptoms, decrease in the size of adnexal masses, and the failure of organisms to grow from menstrual blood, biopsy, or curettage specimens. All patients should be followed by regular pelvic examinations following completion of their treatment, for tuberculosis in the pelvis, like tuberculosis in other organs, is prone to reactivation.

J. Burns Amberson
William R. Barclay
Paul Chapman
Raymond F. Corpe
Felix A. Hughes, Jr.
Carl Muschenheim
Robert L. Yeager
Morris C. Thomas, *Chairman*

REFERENCES

(1) Zummo, B. P., Sered, H., and Falls, F. H.: The diagnosis and prognosis of female genital tract tuberculosis, Am. J. Obst. & Gynec., 1955, *70*, 34.

(2) Sutherland, A. M.: The treatment of tuberculous endometritis with streptomycin and PAS, J. Obst. & Gynec. Brit. Emp., 1954, *61*, 614.
(3) Schaefer, G., and Birnbaum, S. J.: The diagnosis of female genital tract tuberculosis, Obst. & Gynec., 1956, *7*, 180.
(4) Brown, A. B., Gilbert, R. A., and TeLinde, R. W.: Pelvic tuberculosis, Obst. & Gynec., 1953, *2*, 476.
(5) Barnes, T.: The natural history of pelvic tuberculosis, J. Obst. & Gynec. Brit. Emp., 1955, *62*, 162.
(6) Townsend, L.: The diagnosis of pelvic tuberculosis, J. Obst. & Gynec. Brit. Emp., 1955, *62*, 404.
(7) Haines, M.: Genital tuberculosis in the female: I, Obst. & Gynec. Survey, 1953, *8*, 291.
(8) Wahlen, T., Wahlen, L., and Zippis, N.: The interpretation of salpingographic sign of tuberculosis, Acta obst. et gynec. Scandinav., 1955, *34*, 171.
(9) Diagnostic Standards and Classification of Tuberculosis, National Tuberculosis Association, New York, 1955.
(10) Schaefer, G.: Female genital tuberculosis: A review of the literature, Obst. & Gynec. Survey, 1953, *8*, 461.
(11) Clemetsen, N.: Genital tuberculosis with tubercle bacilli in menstrual blood, Acta obst. et gynec. Scandinav., 1947, *27*, 284.
(12) Halbrecht, I.: *Mycobacterium tuberculosis* in uterine discharge: Detection by culture, J.A.M.A., 1950, *142*, 331.
(13) Schaefer, G.: Treatment of female genital tract tuberculosis, Am. J. Obst. & Gynec., 1955, *69*, 1333.
(14) American Trudeau Society: Antimicrobial therapy of tuberculosis, Statement of the Committee on Therapy, Am. Rev. Tuberc., 1955, *72*, 408.
(15) Stallworthy, J.: Genital tuberculosis in the female: II, Obst. & Gynec. Survey, 1953, *8*, 294.
(16) Kullander, S.: Endometrial tuberculosis treated with PAS: Report of a case with subsequent pregnancy, Glasgow M. J., 1952, *33*, 395.
(17) Barnes, T., Smith, H. G. M., and Snaith, L. M.: Isoniazid in the treatment of female genital tuberculosis, Lancet, 1953, *1*, 819.
(18) Hallum, J. L., and Thomas, H. E.: Full-term pregnancy after proved endometrial tuberculosis, J. Obst. & Gynec. Brit. Emp., 1955, *62*, 548.

NOTICES

HARVEY TERCENTENARY CONGRESS, 1957

June 3–June 7, 1957, at the Royal College of Surgeons, London

The tercentenary of the death of William Harvey (1578–1657) will be commemorated by an International Congress on the Circulation from Monday, June 3, to Friday, June 7, 1957, at the Royal College of Surgeons, London, England. It will be followed by a week-end Conference on the more personal and biographical aspects of William Harvey's life at his birthplace, Folkestone, Kent, on Saturday, June 8.

Further details and application for full membership to both the scientific program and social functions may be obtained from: Congress Secretary, 11 Chandos Street, Cavendish Square, London, W. 1, England.

SUMMARY

Potassium para-aminosalicylate (KPAS) was administered to 120 patients with tuberculosis.

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Cohen, R. V.; Molthan, L., and Zarafonetis, C. J. D.: Clinical Studies of Various Forms of PAS (with special reference to plasma concentrations), *Diseases of the Chest* *30*:418-428 (Oct.) 1956.

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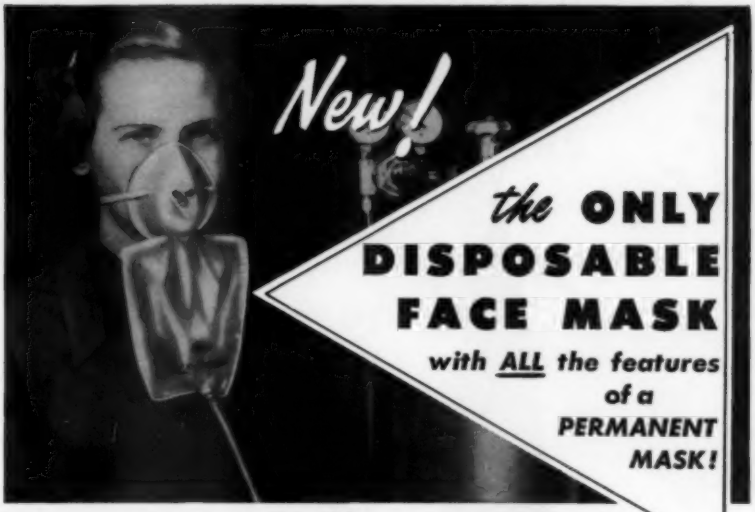

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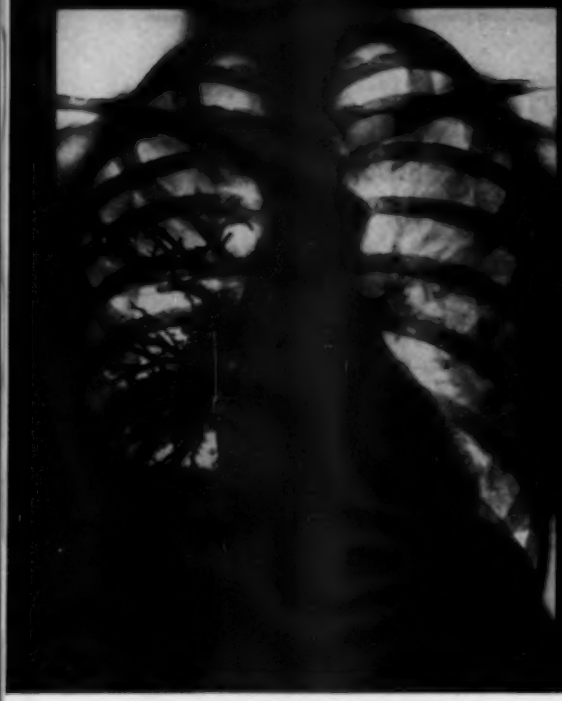

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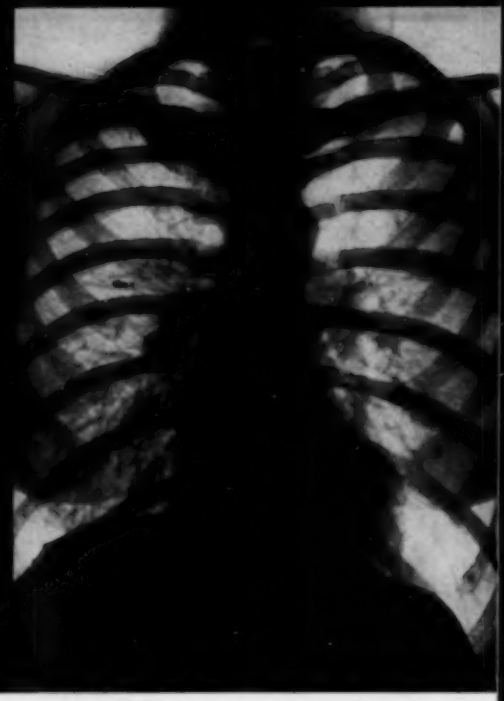

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1. Burrascano, J. J.: Sea View Hosp. Bull. *15*:149 (July) 1955.

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